BEHAVIORAL PREDICTORS OF INDIVIDUAL DIFFERENCES IN OPIOID ADDICTION VULNERABILITY

A DISSERTATION
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

Yayi Swain

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Andrew C. Harris, Ph.D., advisor
Jonathan C. Gewirtz, Ph.D., co-advisor

April, 2021

© Yayi Swain, 2021

Acknowledgements

I feel extremely fortunate to have been able to work with Dr. Andy Harris in his laboratory at Hennepin Healthcare Research Institute as a graduate student. He has been the best primary advisor I could ever hope for, and has provided me with both encouragement and advice throughout my graduate career. I am extremely thankful for him being my role model and showing me how to become a better researcher and scientist. I am also very thankful for my co-advisor Dr. Jonathan Gewirtz, who helped me find this amazing laboratory to work in for my graduate research and guided me along the way as an experienced and knowledgeable mentor.

Thank you to Dr. Niels Waller, who introduced me to the world of multivariate statistics, showed me the importance of conducting rigorous research in psychology, and helped me greatly on my dissertation work. Thank you to all the faculty members at the Department of Psychology and the Department of Neuroscience who taught me many things I had set out to learn at the beginning of my graduate career, including: Dr. Cheryl Olman, Dr. Christopher Federico, Dr. Chun Wang, Dr. Shmuel Lissek, Dr. Eric Newman, Dr. Lucy Vulchanova and Dr. Christopher Honda. I would also like to thank my committee members, Dr. Matt McGue and Dr. Nicola Grissom, for their valuable input on my dissertation and being a part of some of the most memorable moments for me in graduate school.

Thank you to everyone at Hennepin Healthcare Research Institute for the wonderful research environment they established at the laboratories there. Thank you to Dr. Mark LeSage and Dr. Paul Pentel who always had helpful comments for my

presentations at lab meetings. Thank you to Peter Muelken, for patiently teaching me as well as all the undergraduate lab technicians how everything worked in the laboratory and how to work with rats. Thank you to all the technicians who helped with my research projects, including: Annika Skansberg, Danielle Lanzdorf, Zachary Haave, Mary Krueger, Joseph Tombers, Haley Rudnick, and Nettie Enshayan.

Thank you to Yorie Smart, Dr. Tom Molitor, Dr. Marco Pravetoni, and everyone else from the Pharmaco-neuro-immunology training program.

I would also like to thank my wonderful family for always supporting me, and for understanding when I could not visit them back home due to my busy schedule during the past six years. Thank you to many friends I met during my graduate study here at the University of Minnesota, Abbey Hammell, Austin Esser, Ashley Wright, and Dylan Wright, who were there for me during many major milestones and events through my graduate school journey. Thank you to my best friend and life partner Swain San, who supported me in every way possible through all the ups and downs during graduate school, and for always believing in me even during times when I stopped believing in myself.

This dissertation research was supported by by NIH / NIDA grant R21 DA037728 (Gewirtz/Harris, Co-PIs), the Hennepin Healthcare Research Institute (formerly Minneapolis Medical Research Foundation) Translational Addiction Research Program (Harris PI), a Hennepin Healthcare Research Institute Career Development Award for PhD Investigators (Harris PI), and NIDA training grant T32 DA007097 (Swain, Y; Molitor T, PI).

Parts of this dissertation were published previously (full citations below).

Swain, Y., Gewirtz, J. C., & Harris, A. C. (2021). Behavioral predictors of individual differences in opioid addiction vulnerability as measured using IV self-administration in rats. Drug and alcohol dependence, 2021 Apr 1;221:108561. doi: 10.1016/j.drugalcdep.2021.108561.

Swain, Y., Muelken, P., Skansberg, A., Lanzdorf, D., Haave, Z., LeSage, M. G., ... & Harris, A. C. (2020). Higher anhedonia during withdrawal from initial opioid exposure is protective against subsequent opioid self-administration in rats. Psychopharmacology, 237, 2279-2291.

Swain, Y., Muelken, P., LeSage, M. G., Gewirtz, J. C., & Harris, A. C. (2018). Locomotor activity does not predict individual differences in morphine self-administration in rats. Pharmacology Biochemistry and Behavior, 166, 48-56.

Abstract

Understanding behavioral predictors of individual differences in opioid addiction vulnerability could provide critical insights into the mechanisms underlying opioid addiction and could lead to more effective treatments. However, very few behavioral predictors of individual differences in opioid self-administration (SA), a key preclinical model of opioid addiction, have been established. The goal of this dissertation was to evaluate several potential behavioral predictors of individual differences in morphine SA in rats, and to establish novel methodologies for studying opioid addiction vulnerability using the SA paradigm. Study 1 showed that spontaneous locomotor activity in a novel environment, an animal model of sensation-seeking that predicts SA of several drugs of abuse (e.g., stimulants), did not predict individual differences in morphine SA. Study 2 found that greater severity of anhedonia-like behavior during withdrawal from acute morphine exposure (withdrawal-induced anhedonia, WIA) predicted subsequent lower acquisition, demand, and reinstatement of morphine SA. Study 3 showed the feasibility of using regularized factor analysis on morphine SA measures, and revealed that a common latent factor underlies four separate measures of morphine SA. Additionally, while acquisition, demand and morphine-induced reinstatement associated closely with the common latent Addiction factor, stress-induced reinstatement did not. Overall, these studies extended the opioid individual differences literature by establishing WIA as one of the first behavioral predictors of opioid SA, and also expanded the range of analytical tools to be utilized in preclinical behavioral studies.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Characterizing personality and behavioral traits contributing to individual differences in vulnerability to addiction and other psychiatric disorders is essential for developing a greater understanding of underlying genetic and molecular mechanisms, as well as more effective preventions and treatments. Given substantial individual variability in its vulnerability and severity (American Psychiatric Association, 2013; Belin et al., 2016; Vowles et al., 2015), opioid addiction is a prime example of a disorder that could benefit from a clearer understanding of these mechanisms. Despite the enormous toll of opioid addiction on public health, most behavioral predictors of vulnerability to addiction established with other drugs of abuse are less well established when it comes to opioids, both in humans and animal models. Given differences in the neurobiological effects (e.g. receptor pharmacology and drug-induced synaptic and structural plasticity) between different classes of drugs of abuse (Badiani et al., 2011; Ettenberg et al., 1982; Pettit et al.,1984), it is important to characterize vulnerability factors that are specific to opioid addiction.

Preclinical studies provide a number of advantages over human studies for studying individual differences in opioid addiction vulnerability. First, animal behavioral studies allow examination of vulnerability factors in a controlled environment, thereby minimizing the number of extraneous variables (e.g., other mental disorders) that may confound findings. Second, researchers have control over subjects' drug-exposure history, allowing for isolation of the factors uniquely associated with effects of opioids versus other drugs. Third, animal models allow experimental, as opposed to quasi-experimental or cross-sectional study designs, thereby shedding light on causal relationships between variables that could not be identified based on human studies alone. Finally, animal models can utilize invasive techniques to

characterize neurobiological and genetic mechanisms underlying addiction vulnerability (Parker et al., 2014).

In preclinical research, various behavioral models have been developed in an effort to operationalize human personality traits implicated in addiction vulnerability (e.g., impulsivity, novelty-seeking, etc.) The purpose of this review is to evaluate the utility of such measures in predicting opioid addiction vulnerability as measured using the self-administration (SA) paradigm in rats. For several reasons, drug SA is often considered a model with an especially high degree of translational utility. First, while other animal models of addiction (e.g., conditioned place preference, locomotor sensitization) involve experimenter-administered drug, the SA model involves volitional drug-taking, as occurs in human. Second, various SA measures capture different elements of human addictive behavior such as the initiation of drug use (acquisition), loss of control over drug use (escalation), and relapse (reinstatement) (Table 1) (Belin et al., 2008; Grebenstein et al., 2013; McNamara et al., 2010; Leri et al., 2004; Sorge et al., 2005). Moreover, the SA model has some degree of face and predictive validity in modeling opioid addition. For example, there was a close correspondence between the abuse liability of 23 opioid-related drugs in the rat SA model and their positive subjective effects and/or abuse potential in humans (O'Connor et al., 2010). Therefore, despite the limitations in construct validity of any single animal model of human psychopathology (Geyer & Markou, 2000), opioid SA is often considered an appropriate model for studying opioid addiction in animals.

Our primary focus will be on studies of outbred rats, which have been most commonly used and which show significant individual variability in both drug SA itself and in its behavioral

predictors (Parker et al., 2014). Studies of inbred or selectively bred strains will also be discussed to provide further insights on the relationships between certain behavioral predictors and opioid SA propensity. We conclude that few reliable behavioral predictors of opioid SA have been identified. We therefore propose several strategies for assessing and analyzing the relationship between predictor variables and the severity of opioid SA that may help uncover more robust behavioral phenotypes for elucidating the substrates of opioid addiction in people. Such approaches could also help improve the validity and sensitivity of the opioid SA model in general.

Behavioral Predictors of Opioid Addiction Vulnerability

As described below, a number of traits have been evaluated as putative predictors of vulnerability to opioid SA in rats.

Impulsivity

Impulsivity refers to the tendency to engage in premature and suboptimal behaviors (Bardo, 2013; Kurth-Nelson & Redish, 2010). Most facets of impulsivity can be categorized as forms of either impulsive action (difficulty inhibiting or controlling behavior), or impulsive choice (preference for small, immediate rewards over larger, delayed rewards) (Baldacchino et al., 2015; Swann et al., 2009).

Table 1. Measures of SA

Stage of Addiction	**SA Model**	**Operational Measure**	**Example Study**
Initiation of drug use	Acquisition	Average number of infusions earned during first days of drug SA	Belin et al., 2008; Nishida et a., 2016; Smith et al., 2015; Suto et al., 2001
Reinforcing efficacy of drug	Progressive ratio schedule of reinforcement; Behavioral economics	Breakpoint, or the highest fixed ratio at which the animal maintains responding for drug; Elasticity of demand or essential value	Hodos, 1961; Katz, 1990; Richardson & Roberts, 1996; Grebenstein et al., 2013; Stafford et al., 2019
Loss of control over drug use	Escalation	Increase in number of infusions earned after duration of daily access to drug is extended	Kitamura et al., 2006; Edwards & Koob, 2013; Ahmed & Koob, 1999
Drug use despite negative consequences	Resistance to punishment	Reduction in drug SA when infusions are accompanied by aversive consequence (e.g., foot shock)	Deroche-Gamonet et al., 2004 Belin et al., 2008
Relapse to drug use following exposure to drug-associated environmental cues, stress, or the drug itself	Cue-/stress-/drug-induced reinstatement	Increase in drug-seeking (active lever pressing) following extinction of SA and exposure to drug-associated cue stimuli, stress (e.g., foot shock), or non-contingent injection of previously self-administered drug	Childress et al., 1993; Epstein et al., 2006; McNamara et al., 2010; de Wit, 1996; Banna et al., 2010; Sinha, 2001;

Clinical findings

Higher impulsivity has been associated with a higher risk of opioid addiction in some clinical studies (Marino et al., 2013; Nielsen et al., 2012; Vest et al., 2016), while another found no relationship between impulsivity and heroin use (Ahn & Vassileva, 2016). These inconsistent findings may stem from a variety of factors including differences in the population studied, the measure or subscales of impulsivity evaluated (e.g., attentional versus motoric impulsivity), and/or history of opioid and other drug use. Additionally, high impulsivity could be a consequence of opioid exposure rather than a predisposing trait (Baldacchino et al., 2015). As such, the extent to which impulsivity predisposes an individual to opioid addiction is unclear.

Preclinical findings

Preclinical studies have not found an association between trait impulsivity and opioid SA. Individual differences in impulsive action measured using the 5-choice serial reaction time task (5-CSRTT, see Table 2 for a description of this and other behavioral measures evaluated as predictors of opioid SA) did not predict subsequent acquisition, escalation, or cue-induced reinstatement of heroin SA (40 μg/100 μl) under a fixed ratio (FR) schedule of reinforcement in rats (McNamara et al., 2010). Similarly, there was no relationship between impulsive choice in a delayed reward procedure (see Table 2a) and several measures of heroin SA (100 μg/kg/infusion; FR1, 2 and 4) in rats including acquisition, breakpoint during progressive ratio testing (i.e., reinforcing efficacy), or drug seeking during extinction or cue- or drug-induced reinstatement (Schippers et al., 2012). This contrasts with the positive relationships between impulsivity in 5-CSRTT and delayed-reward procedures and SA of other drugs of abuse such as cocaine and nicotine (Belin et al. 2008; Diegaarde et al., 2008; Perry et al., 2005; Anker et

al., 2009). Nevertheless, when rats in the Schippers et al. (2012) study were tested on the delayed-reward task again after completion of heroin SA, those with a history of heroin SA showed increased impulsivity compared to baseline. Another study found no effects of experimenter-administered heroin on impulsivity (Harty et al., 2011). However, a more recent study found that experimenter-administered morphine increased short-term motor impulsivity in adolescent, young adult, and adult rats, and increased long-term motor impulsivity (i.e, following a 25 day drug-free period) in adolescents (Moazen et al., 2018).

Overall, these preclinical studies suggest that impulsive behavior may be an effect of opioid exposure rather than a preexisting vulnerability trait for addiction, as has been suggested in humans (Baldacchino et al. 2015). The findings of Moazen et al. (2018) further suggest that the effects of adolescent opioid exposure on impulsivity may be long-lasting. Such enduring effects could contribute to the difficulty in parsing cause from effect in human studies evaluating the role of impulsivity in opioid addiction vulnerability.

Sensation seeking

Sensation seeking refers to the tendency to attain novel and intense experiences despite risks (Zuckerman, 1994). Sensation seeking has been associated with other addiction-related traits such as impulsivity (Hur & Bouchard,1997; Krueger et al., 2002), and there is some overlap in how these traits are defined (Ahn & Vassileva, 2016; Whiteside & Lynam, 2001).

Clinical findings

Despite some human studies showing a positive relationship between sensation seeking and opioid addiction vulnerability (Franques et al., 2003; Kosten et al., 1994; Vest et al., 2016), others have shown either no relationship (Conrod et al., 2000; Marino et al., 2013; Nielsen et

al., 2012) or a negative relationship (Ahn & Vassileva, 2016). These inconsistencies may reflect the same general limitations of human studies described above.

Preclinical findings

Two tests have been developed to model sensation seeking in rats. The first uses spontaneous locomotor activity in a novel environment as a measure of novelty seeking — a dimension of sensation seeking (Blanchard et al., 2009; Pawlak et al., 2008; Piazza et al., 1989). Higher locomotor activity reliably predicts greater SA of psychostimulants (e.g., cocaine, amphetamine), particularly in terms of acquisition (Piazza et al., 1989; Piazza et al., 2000). These findings are consistent with studies showing a positive relationship between sensation seeking and psychostimulant use in humans (Ahn & Vassileva, 2016; Nielsen et al., 2012).

Only limited data speak to the relationship between spontaneous locomotor activity and opioid SA vulnerability. Inbred rat strains with higher locomotor activity also exhibited greater acquisition of morphine SA (1 mg/kg, FR 1) under certain conditions compared to other strains (Ambrosio et al., 1995). Studies 1 and 2 of this dissertation provide the first characterization of the relationship between locomotor activity and individual differences in opioid SA vulnerability in outbred rats (see below).

Another model for sensation seeking in rats focuses on preference for novelty, measured using a choice task, rather than reactivity to novelty (Belin et al., 2008; Belin et al., 2011; Belin & Deroche-Gamonet, 2012). However, this factor has not been studied in the context of opioid SA vulnerability. Thus, the relationship between opioid SA vulnerability and sensation-seeking, measured using either spontaneous activity or novelty preference, has received only limited attention and requires further elaboration.

Anxiety

Clinical findings

The self-medication hypothesis of addiction (Khantzian, 1987) posits that individuals experiencing greater anxiety are more likely to choose addictive drugs with anxiolytic properties, such as opioids (Khantzian, 1987; Markou et al.,1998). The fact that anxiety has been linked to opioid addiction vulnerability in humans is consistent with this hypothesis (Lejuez et al., 2008; Martins et al., 2012; Norton, 2001; Rogers et al., 2018). However, the experience of negative affect including anxiety is common during withdrawal from opioids and other drugs (Koob & LeMoal, 1997). Therefore, as with other putative predictive traits, it is unclear whether anxiety is a predictor of opioid addiction vulnerability, a consequence of chronic opioid use, or both.

Preclinical findings

The relationship between anxiety and opioid addiction has not been well established in animal models. Rats categorized as showing High- versus Low-anxiety based on time spent in the open arms of an elevated plus-maze (EPM; see Table 2) did not differ in their subsequent escalation of heroin SA (40 μg/100 μl/infusion; FR1), and time spent on the open arms of the EPM did not correlate with heroin SA escalation (Dileen et al 2012). In contrast, anxiety-like behavior in rodents predicts individual differences in SA of drugs other than opioids, such as cocaine (Dilleen et al., 2012; Pelloux et al., 2009; Walker et al., 2009). The role of anxiety in individual differences in opioid SA has not yet been studied via measurement of additional behaviors that more fully capture the multifaceted nature of anxiety and the heterogeneity of

anxiety disorders (e.g., conflict or defensive behavior, conditioned fear) (Blanchard et al., 1993; Shekhar et al., 2001).

Stress Reactivity

Clinical findings

Stress is associated with opioid use both mechanistically and epidemiologically. First, opioids suppress activity of the hypothalamo-pituitary-adrenal (HPA) axis (Goeders, 2007; Facchinetti et a., 1985; Kreek et al., 2005), whereas opioid withdrawal activates the HPA axis (Li et al., 2008). Second, post-traumatic stress disorder (PTSD) and opioid addiction share certain symptoms and are frequently comorbid (Fareed et al., 2013). The link between vulnerability to the effects of stress and to opioid addiction is further supported by clinical studies showing a positive relationship between stress reactivity and opioid use (Back et al., 2015; McHugh et al., 2016). For example, patients with prescription opioid dependence exhibited higher reactivity than controls to acute social stress (Back et al., 2015).

Preclinical findings

Numerous preclinical studies have found that exposure to stressors can elicit or exacerbate opioid addiction-related behavior (e.g., Shaham & Stewart, 1994; Shaham, 1993). However, in the only study to evaluate stress reactivity as a *predictor* of opioid addiction vulnerability (Stafford et al., 2019), higher behavioral (open-field activity, forced swim test) and hormonal (corticosterone) reactivity to intermittent swim stress were predictive of higher reinforcing efficacy for heroin SA (0.05 mg/kg/infusion) in rats measured using a behavioral economic approach (see below for further discussion of behavioral economics). The involvement of the endogenous opioid system in vulnerability to both stress and drug addiction

may underlie this positive relationship (Koob, 2013; Kreek et al., 2005; Piazza & Le Moal, 1996).

Sensitivity to Acute Drug Effects

Clinical findings

Sensitivity to the initial acute effects of drugs (e.g., euphoria, aversion) has long been recognized as a key predictor of addiction vulnerability to drugs other than opioids (DiFranza et al., 2007; O'Loughlin et al., 2003; Schuckit et al., 2004). For instance, sensitivity to the relaxing effects of tobacco during first exposure is a robust predictor of subsequent nicotine addiction (DiFranza et al. 2007). However, these relationships have not yet been examined in clinical studies on opioids.

Preclinical findings

Acute opioid effects

Only one preclinical study has evaluated the relationship between the acute effects of opioids and subsequent opioid SA. That study found that rats with lower sensitivity to the antinociceptive effects of morphine subsequently exhibited greater acquisition of morphine SA at a unit dose of 0.5 mg/kg/infusion under a FR 1 schedule of reinforcement (Nishida et al., 2016). This suggests that reduced sensitivity to the initial analgesic effect of opioids may predict greater opioid addiction vulnerability.

Withdrawal effects

In addition to acute effects, opioid injections can also result in opioid withdrawal in both humans and animals. These withdrawal effects, characterized by negative affective (emotional) states such as anhedonia or diminished reward sensitivity, can be induced after

only a single opioid exposure ("acute" dependence) (Harris & Gewirtz, 2004; Schulteis et al., 2004) and often become more severe with repeated drug exposures (Engelmann et al., 2009; Harris et al., 2004; Schulteis et al., 2004). Avoidance of severe withdrawal effects following prolonged drug exposure may serve as a key motivational force driving compulsive drug-taking (Koob & Le Moal 1997). In contrast, it has been proposed that greater sensitivity to the aversive effects of withdrawal may be a *protective* trait against addiction to opioids and other drugs (Carroll et al., 2008; Dess et al., 2005; Holtz et al., 2015; O'Dell et al., 2006; O'Dell, 2009). Moreover, anhedonia during opioid withdrawal could potentially reduce the motivation for reward-seeking (Wise, 2004). Consistent with these views, saccharin-preferring rats, which exhibit greater SA of opioids and other drugs compared to saccharin non-preferring rats (Carroll et al., 2002), exhibit lower anhedonia during withdrawal from acute morphine injections as measured by increases in intracranial self-stimulation (ICSS) thresholds (i.e., withdrawal-induced anhedonia, WIA). Study 2 of this dissertation evaluates the relationship between WIA and opioid addiction vulnerability in outbred rats (see below).

Contrary to findings using WIA, previous studies have found that saccharin-preferring (i.e., addiction-vulnerable) rats display *higher* anxiety during morphine withdrawal compared to saccharin non-preferring rats as measured by potentiated acoustic startle responding (Radke et al., 2013; Table 2b). In addition, only the saccharin-preferring rats developed a morphine withdrawal-induced conditioned place aversion (Radke et al., 2013). Together, these findings suggest that early-stage negative affective withdrawal signs predict opioid addiction vulnerability, although the direction of the relationship depends on the withdrawal sign measured (e.g., measures of anhedonia vs. anxiety). While these early withdrawal signs could

not be measured in established drug users to predict treatment efficacy, their further investigation in animal models may be valuable in identifying genetic and neurobiological mechanisms underlying vulnerability to opioid addiction.

Conclusion

Evidence for the relationship between specific behavioral indices and opioid addiction vulnerability is mixed in human studies, and sparse in preclinical studies. With the possible exception of sensitivity to agonist/withdrawal effects and stress reactivity, none of the aforementioned behavioral traits reliably predict opioid SA in rats. Furthermore, most of the studies described above used only a single opioid SA unit dose and only one schedule of reinforcement (typically FR 1). These limitations, along with the fact that most of these behavioral phenotypes were exclusively examined in male rats, may limit the generalizability of these findings.

Translation of Preclinical Research

The fact that behavioral traits associated with addiction to other drugs of abuse (e.g., psychostimulants) have not reliably predicted opioid SA in preclinical studies raises the possibility that unique behavioral phenotypes, such as those associated with opioid exposure (e.g., WIA, see above), predict individual differences in opioid SA. It is also possible that other factors implicated in individual differences in vulnerability to SA of other drugs, such as incentive salience (i.e., the tendency to attribute incentive value to drug-associated cues), could prove to be stronger predictors of opioid SA (Beckmann et al., 2011; Flagel et al., 2009). Alternatively, the issue could be one not of searching for additional measures but of refining

Table 2a. Measures of behavioral traits as predictors of opioid SA vulnerability

Predictor	**Behavioral Model**	**Description**	**Study on Opioids**	**Conclusion**
Impulsivity	5-Choice Serial Reaction Time Task (5-CSRTT)	Response to light signal to obtain food after an inter-trial interval. Premature responses are punished	McNamara et al., 2010	No difference between high-and low-impulsivity rats in heroin SA
	Delayed Reward Training	Choice between small, immediate reward or delayed, bigger reward	Schippers et al., 2012	No difference between high- and low-impulsivity rats in heroin SA. Heroin SA increased impulsive responses
Sensation-seeking	Spontaneous Locomotor Activity	Amount of exploratory activity in a novel open-field chamber	Ambrosio et al., 1995	Inbred rat strains with higher activity levels showed greater morphine SA
Anxiety	Elevated Plus Maze (EPM)	Amount of time spent on the open arms (no walls) of the maze	Dilleen et al., 2012	No difference between high-and low-anxiety rats in heroin SA
Stress reactivity	Forced Swim Test (FST)	Number of attempts to climb out of the testing container	Stafford et al., 2019	Climbing behavior positively predicted subsequent demand for heroin SA

Table 2b, Measures of opioid sensitivity as predictors of opioid SA vulnerability

Sensitivity to acute agonist effects	Hot Plate Test (Analgesic Effect)	Nociception after acute opioid injection	Nishida et al., 2016	Lower morphine-induced antinociception predicted greater morphine SA
Sensitivity to acute withdrawal effects	Intracranial Self-Stimulation (ICSS; anhedonia)	Lowest electrical brain stimulation intensity that maintains operant responding	Holtz et al., 2015	Saccharin-preferring rats (high vulnerability) show lower withdrawal-induced anhedonia
	Startle Response (Anxiety)	Acoustic startle response during withdrawal	Radke et al., 2013	Saccharin-preferring rats exhibit higher anxiety during withdrawal
	Conditioned Place Aversion (CPA)	Amount of time spent in an environment associated with withdrawal	Radke et al., 2013	Only saccharin-preferring rats develop CPA to morphine withdrawal

existing ones. That is, our metrics for measuring addiction vulnerability using opioid SA, and our means of statistically evaluating their relationship to specific vulnerability factors, may underestimate those factors' predictive value. We propose that employing one or more novel approaches to behavioral measurement and statistical analysis, some of which have already shown their worth in human studies, may enable us to establish relationships between predictors and outcomes of opioid SA with greater construct and face validity (Geyer & Markou, 1995; Markou et al. 2009; Smith, 2020). These approaches may ultimately also prove beneficial in furthering our understanding of individual differences in addiction to other drugs of abuse, such as stimulants.

Further Refining the SA Paradigm

Behavioral Economics

Behavioral economics quantifies the extent to which consumption of a reinforcer (e.g., drug) is maintained following increases in its "unit price." In drug SA models, unit price is operationalized as the cost-benefit ratio of response requirement and unit dose (Bickel et al., 2000; Hursh, 1991; Hursh & Silberberg, 2008). A more rapid decrease in consumption following increases in unit price (lower demand) indicates lower abuse liability, while a slower decrease (higher demand) indicates higher abuse liability. Behavioral economics provides an operationalized and quantifiable measure of reinforcement efficacy that can be used in humans and animals, and has been useful for studying individual differences in demand for numerous addictive drugs (e.g., nicotine) in both species (Diergaarde et al., 2008; Grebenstein et al., 2013; Chase et al., 2013; Hursh & Silberberg, 2008).

A growing body of clinical and preclinical evidence supports the utility of behavioral economics in the study of opioid addiction. For instance, greater demand predicted poorer treatment outcomes for prescription opioid dependence (Worley et al., 2015). Additionally, an exponential demand function closely approximated opioid demand in current and previous opioid users (Strickland et al., 2019), as well as in animals (Stafford et al., 2019). A recent preclinical studyexamined the relationships between stress reactivity and demand, demonstrating the feasibility and utility of this analytical approach in preclinical opioid addiction research (Stafford et al., 2019). Since most rodent opioid addiction measures are not directly comparable to measures used in humans, behavioral economics could prove to be a powerful translational tool (Bentzley et al, 2013).

Alternative Addiction Models

Developing behavioral measures that more closely resemble those used to define addiction in humans may also be useful for establishing reliable predictors of opioid SA in preclinical models (Belin-Rauscent et al., 2016). Much as the Diagnostic and Statistical Manual of Mental Disorders (DSM) is utilized to measure addiction in humans (American Psychiatric Association, 2013), Belin and colleagues proposed a checklist approach to classifying addiction behavior in rats (Belin & Deroche-Gamonet, 2012). Three measures of drug SA were selected, each corresponding to a DSM diagnostic criterion, with the total score taken as the addiction score. In theory, other criteria, such as tolerance and withdrawal severity, could be similarly incorporated into such a model.

Alternatively, a number of researchers have modeled addiction in rodents by construing it as a disorder of “choice”; namely, the choice between drugs and non-drug

reinforcers such as social interaction, financial stability, etc. (Heyman, 2009; Townsend et al., 2019). This approach emphasizes the availability of non-drug rewards that compete with drugs for the individual's attention and efforts to obtain. With the flexibility of SA, one can establish reliable procedures to assess individual differences in choice between drug and food or between drug and social interaction (Banks & Negus, 2017; Venniro & Shaham, 2020). In a recent study utilizing these procedures, Townsend and colleagues found that although female rats self-administered more fentanyl than males when it was the only reinforcer, males self-administered more fentanyl when the drug was available with an alternative food reinforcer (Townsend et al., 2019). By establishing choice procedures and examining the relationship between various predictors of individual differences in choice between drug and ethologically valid, non-drug reinforcers, researchers could add to the existing preclinical literature that views addiction primarily as a disorder of disinhibition (Belin et al., 2016; Townsend et al., 2019; Smith & Pitts, 2012).

Multivariate Designs and Statistics

Utilizing Multivariate Designs in Addiction Research

Studies in which more than one outcome is simultaneously observed and analyzed provide unique information about the clustering and interactions of different factors contributing to the behavioral outcomes of interest. Human studies have long incorporated multivariate designs and corresponding statistical methods to reveal complex relationships between large numbers of predictors and outcome measures of addiction (Krueger et al., 2002; Ahn & Vassileva, 2016; Lynskey & Agrawal, 2007). For example, using elastic net regression, a machine-learning multivariate statistical method, Ahn and Vassileva (2016)

identified distinct groups of personality traits associated exclusively with amphetamine or heroin use.

Multivariate study designs also allow us to examine the potential role of a reduced set of unobservable "latent" variables in accounting for variability among a much larger number of related variables. Such variables have greater statistical reliability than individual measures and have been valuable in characterizing core dimensions contributing to addiction vulnerability (Krueger et al., 2002; Lynskey & Agrawal, 2007; Monga et al., 2007). In the human literature, some exploratory factor analysis (EFA) studies have suggested a single latent variable underlying many aspects of opioid addiction (Lynskey & Agrawal, 2007), while others have indicated 2 or 3 factors, each underlying different aspects of the disorder (Monga et al., 2007).

In contrast to EFA, confirmatory factor analysis (CFA) provides a method for testing a priori theory-driven models of factor structure (Schmitt, 2011). This approach has shown that alcohol abuse risk reflects both a general liability to abuse of differing substances along with distinctive influences that give rise to alcohol abuse specifically (e.g., genetic variants in alcohol metabolizing enzymes) (Krueger et al., 2002; Tsuang et al., 1998; Luczak et al., 2006). By adopting EFA and CFA methods, preclinical studies could complement human opioid addiction research in identifying dimensions underlying addiction vulnerability and their biological substrates.

Latent variable analyses would also help in improving the ability of animal models to capture important facets of human behavior and psychopathology. Most animal studies select one or two variables to examine while controlling all others. This approach provides

important insight on cause and effect. However, broader application of such insights may be limited by sources of variability specific to each individual trait measure or outcome measure. Such idiosyncrasies may be associated with a specific measure or paradigm (e.g. speed of learning in a conditioning paradigm), or the manner in which that paradigm is conducted in a specific lab. This variability can make it difficult to generalize across different preclinical studies, or from preclinical to clinical studies. Since latent variables capture only the commonality between a number of observed measures (e.g., demand and choice, measured in the same animals), they are relatively impervious to the idiosyncratic and unique variability of each observed measure. Therefore, latent variable analyses can provide more robust information about the relationships between observed measures and underlying constructs.

Multivariate Designs in Preclinical Studies

A recent study used linear mixed-effects modeling to determine the predictability of demand for heroin as a function of one or multiple stress reactivity measures (Stafford et al., 2019). The results showed that models including multiple predictors explained a greater proportion of variance in heroin demand than did any bivariate model, demonstrating the value of incorporating multiple measures. Linear model and factor analytic approaches have begun to be used to identify predictors of individual differences in psychostimulant SA (e.g., Dickson et al., 2015; Marusich et al., 2011; Belin et al., 2008; Deroch-Gamonet et al., 2014). Nevertheless, multivariate designs and statistical methods have been underutilized in preclinical studies of addictive drugs in general and of opioids in particular. In addition to conducting *a priori* multivariate analyses on novel datasets,

existing or published datasets could also be re-analyzed with multivariate statistics when applicable, to further establish latent factors underlying addiction severity, as well as help identify clusters of behavioral traits that are most closely associated with these latent factors.

Small Sample Size Multivariate Analyses

One of the biggest challenges to incorporating multivariate statistics methods in preclinical research is a common reliance on small sample sizes. Estimates for the minimum sample size required for a factor analysis have ranged widely e.g., N = 100-250 (Cattell, 1978; Gorsuch, 1983; Guilford, 1954; Kline, 1979). Most of these recommended sample sizes are impractical for preclinical behavioral studies. This may be a primary reason why between-group approaches have been favored over within-subject, factor-analytic approaches in the preclinical literature. It is encouraging, therefore, to note the incorporation of much larger sample sizes (e.g., >1000 rats) in several recent or ongoing studies in outbred Heterogeneous Stock (HS) rats in order to conduct genomic analyses of complex traits (Gileta et al., 2018; Hughson et al., 2019).

While such studies are unlikely to become commonplace, it is now feasible with advances in quantitative psychology and statistics to conduct factor analysis and structure equation modeling with much smaller sample sizes. For example, the regularization method involves shrinking or adding penalties to specific parameters within the statistical models. The viability of this approach for conducting factor analysis and structure equation modeling using small sample sizes has been demonstrated in both simulation and human studies (Jacobucci et al., 2016; Jung & Takane, 2007; Jung & Lee, 2011). In fact,

regularization provides reasonable factor recovery (i.e., how close the sample factor loadings are to population factor loadings) with sample sizes as small as N=5 and N=10 (albeit, less than can be achieved with much larger sample sizes), and yields stable factor loadings when the number of measured variables is large (Jung & Lee, 2011). Greater use of these methods could enable preclinical studies with smaller sample sizes to implement complex models and test hypotheses more comparable and relevant to those tested in clinical addiction research.

Conclusions

Despite the prevalence of opioid addiction and the societal burden it imposes, few factors have been associated with opioid addiction vulnerability in humans or animals. By utilizing more clinically relevant measures of drug addiction, and by further adopting and adapting data analytic tools commonly used in human studies, preclinical behavioral studies may further increase the construct and predictive validity of opioid SA and provide new insights into factors associated with vulnerability to opioid addiction, their genotypic and neurobiological basis, and their potential role in prevention and treatment.

The goal of this dissertation was to provide novel insights into individual differences in behavioral predictors of opioid addiction vulnerability. Studies 1 and 2 (see Chapters 2 & 3) examined two potential behavioral predictors of opioid addiction vulnerability, using a variety of morphine SA measures, while Study 3 (see Chapter 4) adapted novel statistical methods to explore the latent factor structure underlying morphine SA measures.

Chapter 2: Locomotor Activity Does Not Predict Individual Differences in Morphine Self-administration in Rats

Opioid addiction poses a tremendous burden on public health (Center for Behavioral Health Statistics and Quality, 2013; Center for Behavioral Health Statistics and Quality, 2015). Although many people experiment with opioids, only a minority undergo the loss of control over drug use that defines addiction (American Psychiatric Association, 2013; Belin et al., 2016). Understanding the behavioral and neurobiological mechanisms contributing to individual differences in opioid addiction vulnerability is essential for developing more effective preventions and treatments.

Sensation-seeking, or the pursuit of novel and intense experiences and a willingness to take risks in order to attain such experiences (Zuckerman, 1994), has been implicated in vulnerability to addiction to a variety of drugs including stimulants (e.g. cocaine, amphetamine) and alcohol (Bardo et al., 2013; Belin et al., 2016; Hittner & Swickert, 2006; Piazza et al., 1989). However, the relationship between sensation-seeking and opioid addiction vulnerability has not been well established. Some studies have shown a positive relationship between sensation-seeking and opioid use in humans (Franques et al., 2003; Kosten et al., 1994; Vest et al., 2016), while others have shown either no relationship (Conrod et al., 2000; Marino et al., 2013; Nielsen et al., 2012) or a negative relationship (Ahn & Vassileva, 2016). The reasons for these discrepancies across studies are unclear, but may reflect differences in subject characteristics (e.g., age, sex, drug use history, and/or comorbidities), measure(s) of sensation-seeking, or other factors (Marino et al., 2013).

Animal models allow for greater experimental control than human studies, and

could be useful for understanding the role of sensation-seeking in opioid addiction vulnerability. Spontaneous locomotor activity in a novel environment is a commonly used preclinical model of sensation-seeking (Blanchard et al., 2009; Pawlak et al., 2008; Piazza et al., 1989). Consistent with the relationship between sensation seeking and stimulant use in humans, higher activity reliably predicts greater self-administration (SA) of stimulants (e.g., cocaine, amphetamine), particularly in terms of acquisition (Belin et al., 2008; Belin et al., 2011; Belin & Deroche-Gamonet, 2012; Piazza et al., 1989; Piazza et al., 2000).

Only limited data are available regarding the relationship between spontaneous locomotor activity and individual vulnerability in i.v. opioid SA. In a comparison between several inbred rat strains, those strains with higher activity levels also exhibited greater acquisition of morphine SA under certain conditions (Ambrosio et al., 1995; see Discussion for further details). However, the relationship between locomotor activity and opioid SA in outbred rodents has not been evaluated. This represents an important research gap given that inbred and outbred rats are genetically distinct, and because findings on predictors of addiction vulnerability in inbred and outbred rat strains are not always concordant (Cadoni et al., 2015; Chaouloff et al., 1995; Dilleen et al., 2012; Meyer et al., 2010).

The primary goal of this study was to evaluate locomotor activity as a predictor of individual differences in the acquisition of morphine SA in outbred rats. Because activity did not predict acquisition of morphine SA under the conditions initially studied (0.5 mg/kg/infusion, 4 hr/day sessions), we evaluated the generality of this finding to a different model with a lower dose and shorter access period (0.2 mg/kg/infusion, 2 hr/day sessions). This approach was used because the relationship between activity and SA of other drugs

(e.g. cocaine) can be more apparent when lower unit doses and/or shorter access periods are used (Belin et al., 2016; Kabbaj, 2006; Mantsch et al., 2001).

A secondary goal was to apply a behavioral economics framework to the analysis of individual differences in morphine SA. Behavioral economics involves evaluation of the extent to which consumption of a reinforcer (e.g., drug) is maintained following increases in its unit price, which in drug SA models is operationalized as the cost-benefit ratio of response requirement/unit dose (Bickel et al., 2000; Hursh, 1991; Hursh & Silberberg, 2008). Behavioral economics has been useful for studying individual differences in elasticity of demand (i.e., reinforcing efficacy) of numerous addictive drugs (e.g., cocaine) in both humans and animals (Diergaarde et al., 2008; Grebenstein et al., 2013, Hursh & Silberberg, 2008, LeSage et al., 2016), but has not yet been applied to morphine SA in rodents. Therefore, we evaluated elasticity of demand in animals that acquired morphine SA in both experiments in order to 1) evaluate the precision and generalizability of a behavioral economic framework in the context of morphine SA, and 2) provide a preliminary evaluation of the relationship between locomotor activity and individual differences in behavioral economic measures.

Materials and Methods

Animals

Male adult Sprague Dawley rats (Envigo, Indianapolis, IN) weighing 276-300 g at arrival were used. All rats were individually housed in a temperature- and humidity-controlled colony room with unlimited access to water under a reversed 12-h light/dark cycle (lights off at 10:00 hr). All behavioral testing occurred during the dark (active) phase.

Beginning one week following arrival, food was restricted to 18 g/day to facilitate operant performance, avoid detrimental health effects of long-term ad libitum feeding, and limit catheter migration. Protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation in accordance with the 2011 National Research Council's Guide for the Care and Use of Laboratory Animals and the 2003 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

Apparatus

Locomotor Activity

Locomotor activity was monitored in 43 x 43 cm open field activity chambers (Med Associates, Inc., St. Albans, VT). Each chamber had two 16-beam photocell arrays placed 5 cm and one array 18 cm above the chamber floor to monitor horizontal and vertical activity, respectively. Chambers were placed inside sound-attenuating cubicles equipped with exhaust fans that provided masking noise and ambient lighting. Open-field activity software (Med Associates) was used for operating the apparatus and recording data.

Morphine Self-Administration

Self-administration (SA) sessions were conducted using 16 standard operant conditioning chambers (model ENV-007, Med Associates, Inc). Each chamber contained two response levers, a white stimulus light located 2 cm above each lever, and a house light that provided ambient illumination. Each chamber was placed inside a sound-attenuating cubicle equipped with an exhaust fan that provided masking noise. An infusion pump (model PHM-100-15, Med Associates) placed outside each cubicle delivered infusions at

a rate of 100 µl/kg per second. MED-PC IV software (Med Associates) was used for operating the apparatus and recording data.

Drugs

Morphine sulfate (NIH National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) was dissolved in sterile saline and heparin (30 units/ml) was added to maintain catheter patency. Morphine doses are expressed as the weight of the salt.

Surgical Procedures

Each rat was implanted with a chronic indwelling catheter into the right jugular vein under isoflurane (1%-3%) anesthesia, using general surgical procedures described in detail elsewhere (Harris et al, 2008; LeSage et al, 2002). The catheter was externalized between the scapulae and attached to A vascular-access harness (VAH95AB, Instech Laboratories, Plymouth Meeting, PA) that allowed connection to a fluid swivel via a tether for morphine administration. Animals were allowed to recover for one week after surgery, during which time they received daily i.v. infusions of heparinized saline, ceftriaxone antibiotic (5.25 mg, first three days only), and s.c. injections of buprenorphine (0.05 mg/kg; first two days only) for analgesia. Infusions of methohexital (0.1 ml, 10 mg/ml, i.v.) were administered to check patency post-session on Fridays throughout all protocols. If a catheter became occluded (indicated by a failure of the animal to exhibit anesthesia within 3-5 sec after methohexital infusion), another catheter was implanted into the ipsilateral femoral vein. Failure of this second catheter resulted in removal of the animal from the study.

Experimental Protocols

Experiment 1

Six days after arrival, rats (N = 16) were monitored for locomotor activity in a novel open field for 2 hours. At least 24 hours later, rats were catheterized as described above. After a 7-10-day recovery period, rats were allowed to acquire i.v. morphine SA during daily 4 hr sessions conducted Mon-Fri. During each session, responding on the left ("active") response lever resulted in an i.v. infusion of morphine sulfate at a unit dose (0.5 mg/kg/infusion) that maintains robust SA and that has previously been used to evaluate other determinants (e.g., pain sensitivity) of individual differences in morphine SA (Park et al., 2012; Nishida et al., 2016). Each infusion was accompanied by offset of the house light and the onset of a white cue light above the active response lever. Following a 5-second timeout period, the cue light above the active lever was extinguished to signal availability of the next infusion. Responses on the other lever in the operant chamber (the "inactive" lever) were recorded but had no programmed consequences. On the first day of acquisition (always a Friday), food powder was placed on the active lever to facilitate contact with the lever. Data from this session were not included in the data analysis. Beginning the following Monday, rats were tested under an FR 1 schedule for at least 10 sessions and until acquisition criteria were met (≥ 5 infusions per session, ≤20% coefficient of variation, and ≥ 2:1 response ratio on the active to inactive lever) across 3 sessions, at which point the FR was increased to FR 2 for at least 5 sessions and then to FR 3. We used a FR 3 schedule prior to unit dose manipulation in order to be consistent with previous studies evaluating elasticity of demand for other drugs (e.g, nicotine) in our lab (Grebenstein et al., 2013; Grebenstein et al., 2015; Raleigh et al., 2014). When infusion

rates were stable under the FR 3 schedule (same stability criteria as above), unit price was manipulated by progressively reducing the morphine unit dose according to the following progression: 0.3, 0.1, 0.05, 0.025, 0 mg/kg. Each unit dose was tested for 5 sessions. Four rats exhibited self-mutilation and were tested under an accelerated schedule in order to expedite the protocol. These animals were tested in 1-3 sessions per unit dose at either 1 (n = 3) or 2 (n = 1) of the 6 unit doses. All of these animals completed the standard 5 sessions per unit dose for the remaining unit doses. Data for these 4 animals did not impact our overall conclusions and are included in the analyses below.

Experiment 2

Rats (*N* = 22) were tested for locomotor activity as described in Experiment 1, catheterized, and allowed to acquire i.v. morphine SA at a unit dose of 0.2 mg/kg/infusion (n = 16) or 0 mg/kg/infusion (saline) (n = 6) in 2 hour/day sessions under a FR 1 schedule for at least 10 sessions. A longer timeout period following each infusion (30 sec rather than 5 sec) was used in an attempt to avoid the self-mutilation observed in Experiment 1. No mutilation was observed at any point in this experiment. In animals exhibiting stable SA at FR 1 (same criteria as above), the FR was increased every 5 sessions according to the following progression: FR 2, 3, 6, 12, 24, etc., until infusion rates declined by at least 90% compared to FR1. Unit price was manipulated via increases in FR requirement rather than decreases in unit dose for two reasons. First, based on data from Experiment 1, it was unclear whether a dose-reduction protocol using a 0.2 mg/kg/infusion training dose would provide a sufficient number and range of unit prices for a behavioral economic analysis (see Fig 1D). This approach also allowed us to evaluate the precision of the exponential

demand function for describing morphine consumption when unit price was manipulated in this manner. In theory, increasing unit price via dose reduction or FR escalation should produce functionally equivalent effects on consumption (Bickel et al., 1995; Greenwald & Hursh, 2006).

Statistical Analyses

All statistical analyses and graphing were performed either in GraphPad Prism 7 or R (ver. 3.2.3). Locomotor activity was measured as total distance traveled (in cm) during the 2-hour activity test and was analyzed using one-way ANOVA. Secondary measures of activity included ambulatory count (horizontal photobeam breaks), stereotypic count (repetitive horizontal photobeam breaks) and vertical count (vertical photobeam breaks). In general, lever presses or infusions during morphine SA were analyzed using ANOVA followed by Sidak's or Dunnet's multiple comparison tests (see Results for more details). The primary measure of morphine SA acquisition was the mean number of infusions per session during the first 10 days of acquisition. Secondary measures of acquisition were 1) mean number of infusions during the first 10 minutes of each session during the first 10 days of acquisition, a period that can be especially sensitive for detecting predictors of morphine SA (Nishida et al., 2016), and 2) number of days to achieve acquisition criteria. Pearson's correlation was used to examine relationships between locomotor activity and measures of morphine SA acquisition and demand (see below). To confirm that collapsing locomotor data across the entire 2 hour locomotor session did not obscure a relationship between early-session activity and morphine SA, these correlational analyses were repeated using locomotor activity during only the first 30 minutes of the session. Finally,

in an additional analysis, measures of morphine SA were compared between subgroups of rats designated as high or low responders based on whether their 2 hour activity level was above or below the median of the sample (see Dellu et al., 1996; Piazza et al., 1989; Piazza et al., 2000). In both experiments, these comparisons yielded the same conclusions as the correlational analyses and are not reported.

To determine elasticity of demand (reinforcing efficacy) during unit dose reduction (Experiment 1) or FR escalation (Experiment 2), exponential demand curve analyses were conducted using the following equation:

$$Log\ Q = LogQ_0 + k(e^{-\alpha \cdot Q_0 \cdot C} - 1)$$

In this model, Q is the quantity consumed. The independent variable, C, is the cost of morphine based on the unit price (FR/unit dose). The free parameters, Q_0 and α are estimated from the best-fit function and refer to the maximum level of consumption at zero price (i.e., level or "intensity" of demand) and the rate of change in consumption with increases in unit price, respectively. The range of the exponential function, k is a constant specifying the range of consumption in log units. The k value is held constant across all data sets being compared in each experiment (set to 2.3 in Experiment 1 and 1.8 in Experiment 2), because changes in k impact the value of α. The α parameter is considered a measure of reinforcing efficacy, such that drugs that produce rapidly declining (elastic) demand curves have higher α values and lower reinforcing efficacy than demand curves with slower declining (inelastic) demand curves. Therefore, α served as the index of elasticity of the demand for, or the reinforcing efficacy of morphine. Other demand measures of interest included: Q_0, the level or intensity of demand as described above;

Omax, the maximal response output; and Pmax, the unit price at which maximal response output occurred. Demand functions were generated using a template for GraphPad Prism software provided by the Institutes for Behavior Resources, Inc. (Baltimore, MD) on their website.

Results

Experiment 1

Locomotor Activity

One-way ANOVA of locomotor activity in rats that later completed at least 10 days of morphine self-administration (SA) indicated a main effect of time ($F(6.939, 104.1) = 43.35$, $p < 0.0001$). Activity levels were highest during the first 30 minutes of the 2-hour session (Fig 1A), consistent with previous literature (e.g., Piazza et al., 1989).

Acquisition

All data were removed for one rat that pulled out its catheter following 8 acquisition sessions and died during i.v. catheter re-implantation. A two-factor ANOVA on data for the remaining 15 animals indicated a significant main effect of lever ($F(1, 14) = 119.2$, $p<0.0001$), but no main effect of session or interaction during the first 10 acquisition sessions (Fig 1B). Sidak's multiple comparisons test further showed significant differences in responding on the active versus inactive levers during all 10 sessions ($t = 5.24 – 6.93$, $p<0.0001$). Infusion rate did not differ significantly across sessions (Fig 1C).

Elasticity of Demand

Table 3.

Exponential demand curve parameters for individual subjects

Subject	α	Q_0	P_{max}	O_{max}	R^2
Experiment 1					
1	0.00046	23.0	22.8	168.5	0.89
2	0.00057	17.0	24.9	136.0	0.91
3	0.00078	14.0	22.1	99.4	0.83
4	0.00060	9.9	40.6	129.2	0.95
5	0.00070	14.0	24.6	110.8	0.85
6	0.00048	13.0	38.6	161.5	0.96
7	0.00079	9.9	30.8	98.1	0.98
8	0.00290	1.7	48.9	26.7	0.81
9	0.00078	15.0	20.6	99.4	0.98
10	0.00100	12.0	20.1	77.5	0.96
11	0.00320	2.6	29.0	24.2	0.89
12	0.00092	13.0	20.2	84.3	0.96
13	0.00100	17.0	14.2	77.5	0.92
14	0.00130	19.0	9.8	59.6	0.97
Mean	**0.00111**	**12.94**	**26.23**	**96.62**	**0.92**
SEM	**0.00229**	**1.54**	**2.83**	**11.62**	**0.02**
Experiment 2					
1	0.00160	6.8	29.7	64.3	0.65
2	0.00650	12.0	4.1	15.8	0.83
3	0.00150	4.7	45.8	68.6	0.85
4	0.00098	6.2	53.1	105.0	0.92
5	0.00240	4.8	28.0	42.9	0.77
6	0.00140	8.4	27.4	73.5	0.92
7	0.00058	11.0	50.6	177.4	0.91
8	0.00076	3.5	121.3	135.4	0.85
Mean	**0.00197**	**7.18**	**45**	**85.36**	**0.84**
SEM	**0.00068**	**1.08**	**12.27**	**18.36**	**0.03**

Note. The parameter k is set to 2.3 log units for experiment 1 and 1.8 log units for experiment 2.

One rat did not complete dose-response testing due to multiple catheter occlusions. Data for this animal have been removed. One-way ANOVA on data for the remaining animals revealed a significant effect of dose on number of infusions ($F(1.786, 23.21) = 19.91$, $p<0.0001$). Post-hoc Dunnett's multiple comparisons test showed that infusions at 0.05, 0.1, 0.3, 0.5 mg/kg/infusion were all significantly higher than at 0 mg/kg/infusion ($q = 4.41 – 6.35$, $p < 0.0001$-0.003), while there was only a marginally significant difference between infusions at 0.025 versus 0 mg/kg/infusion ($q = 2.69$, $p= 0.0686$, see Fig 1D).

Morphine consumption during demand testing was well-described by an exponential demand function, with R^2 values typically ≥ 0.85 for individual animals and $R^2 = 0.97$ for rats as a group (Table 3, Figure 1E). There was a considerable degree of individual variability in α values (i.e., elasticity of demand), with some rats showing a rapid decline in morphine consumption following increases in unit price (i.e., reductions in unit dose) (e.g., rat #14 in Table 3 and Fig 1E) and others maintaining significant consumption despite the increases in unit price (e.g., rat #6 in Table 3 and Fig 1E).

Correlations

Pearson's correlation indicated that total distance traveled during the 2-hour locomotor test was not correlated with average daily infusion rate during acquisition of morphine self-administration (SA) ($r = 0.05$, $p = 0.86$), average infusions during the first 10 minutes of each session during acquisition ($r = -0.21$, $p = 0.46$) (Figure 2A-B), or days to acquire ($r = 0.23$, $p = 0.41$) (Figure 2C; $n = 15$). Moreover, distance traveled during locomotor testing did not predict elasticity of demand (reinforcing efficacy) ($r = -0.11$, $p =$

0.70) (Figure 2D), intensity of demand (maximum consumption at zero price) (r = 0.10, p = 0.73) (Figure 2E), Pmax (r = 0.14, p = 0.63), or Omax (r = 0.18, p = 0.55) (data not shown graphically; n = 14). Consistent with the above analyses, activity during the first 30 minutes of the 2 hour locomotor test was not correlated with average daily infusion rate during acquisition (r = 0.31, p = 0.26) or any other measure of morphine SA (data not shown). Secondary activity measures including ambulatory count (mean ± SEM = 2027.7, SEM = 181.1), stereotypic count (12403.3,± 702.1) and vertical count (208.7 ± 21.5) were not significantly correlated with average daily infusion during acquisition or other measures of morphine SA (all p ≥ 0.33) (data not shown).

Experiment 2

Locomotor Activity

As in Experiment 1, one-way ANOVA of locomotor activity indicated a main effect of time ($F(7.058, 105.9) = 69.27$, $p < 0.0001$), with most activity occurring during the first 30 minutes of the 2-hour session (Fig 3A).

Acquisition

A three-factor ANOVA (lever x drug x session) indicated significant main effects of lever (active vs inactive) ($F(1, 20) = 10.70$, $p=0.004$) and drug (morphine vs. saline) ($F(1, 20) = 10.25$, $p=0.004$), and a significant lever x drug interaction ($F(1, 20) = 5.14$, $p=0.035$). There was no main effect of session or other interactions. A two-factor ANOVA on data for the morphine group indicated a significant effect of lever ($F (1, 15) = 22.16$, $p=0.0003$), but no effect of session or interaction (Fig 3B). Sidak's multiple comparisons test further showed significant differences in responding on the active versus inactive

levers during all 10 sessions ($t = 3.33 – 6.46$, $p<0.01$). Analysis of active and inactive lever data for rats in the saline group indicated no effect of lever, session, or interaction.

Analysis of infusion rates indicated a significant effect of drug ($F (1, 20) = 11.36$, $p=0.0030$), but no effect or session or interaction (Fig 3C).

Elasticity of Demand

None of the animals in the saline group were tested for demand because none of them achieved SA acquisition criteria. Eight of the 16 animals in the morphine group did not complete this phase due to failure to acquire, loss of catheter patency, or other problem. One-way repeated measures ANOVA on data from the 8 remaining animals revealed significant effect of FR on number of morphine infusions ($F (1.941, 15.53) = 21.29$, $p<0.0001$). Post-hoc Dunnett's multiple comparisons test showed that infusions at FR 12, 24 and 48 were all significantly lower than at FR 1 ($q = 3.94 – 6.62$, $p = 0.02$-0.0007). Infusions at FR 6 only marginally differed from infusions at FR 1 ($q = 3.00$, $p= 0.07$) (Fig 3D).

Morphine consumption during demand testing was generally well-described by an exponential demand function ($R^2 = 0.96$ for rats as a group, Fig 3E), with considerable individual variability in α values (Table 3).

Correlations

Distance traveled during the 2-hour locomotor test was not correlated with average daily infusion rate during acquisition of morphine SA ($r = 0.06$, $p = 0.82$, $n = 16$), average infusions during the first 10 minutes of each session ($r = -0.16$, $p = 0.56$, $n = 16$) (Figure 4A-B), or days to acquire ($r = -0.11$, $p = 0.73$, $n = 13$) (Figure 4C). Moreover, distance

traveled did not predict elasticity of demand ($r = -0.21$, $p = 0.62$) (Figure 4D), intensity of demand ($r = -0.13$, $p = 0.77$) (Figure 4E), Pmax ($r = 0.30$, $p = 0.47$), or Omax ($r = 0.20$, $p = 0.64$) (data not shown graphically; $n = 8$ for all behavioral economic measures). Activity during the first 30 minutes of the 2 hour locomotor session also was not correlated with average daily infusion rate during acquisition ($r = 0.002$, $p = 0.99$) or any other measures of morphine SA (data not shown). Other measures including ambulatory count (mean $\pm$ SEM = 2640.9, 177.5), stereotypic count (14046.4 $\pm$ 755.6) and vertical count (228.3 $\pm$ 15.7) were not correlated with average daily infusion during acquisition or other measures of morphine SA (all $p \geq 0.25$) (data not shown).

Figure 1. (A) Mean (± SEM) distance traveled in 5-minute blocks during locomotor testing. Mean (± SEM) number of response on the active and inactive levers (B) and infusions per session (C) during acquisition in Experiment 1. (D) Mean (± SEM) number of infusions at each morphine SA unit dose during demand testing. (E) Exponential demand curve describing morphine consumption as a function of unit price for rats as a group. Demand curves for individual rats with relatively low (rat #6) and high (rat #14) elasticity of demand are also shown.

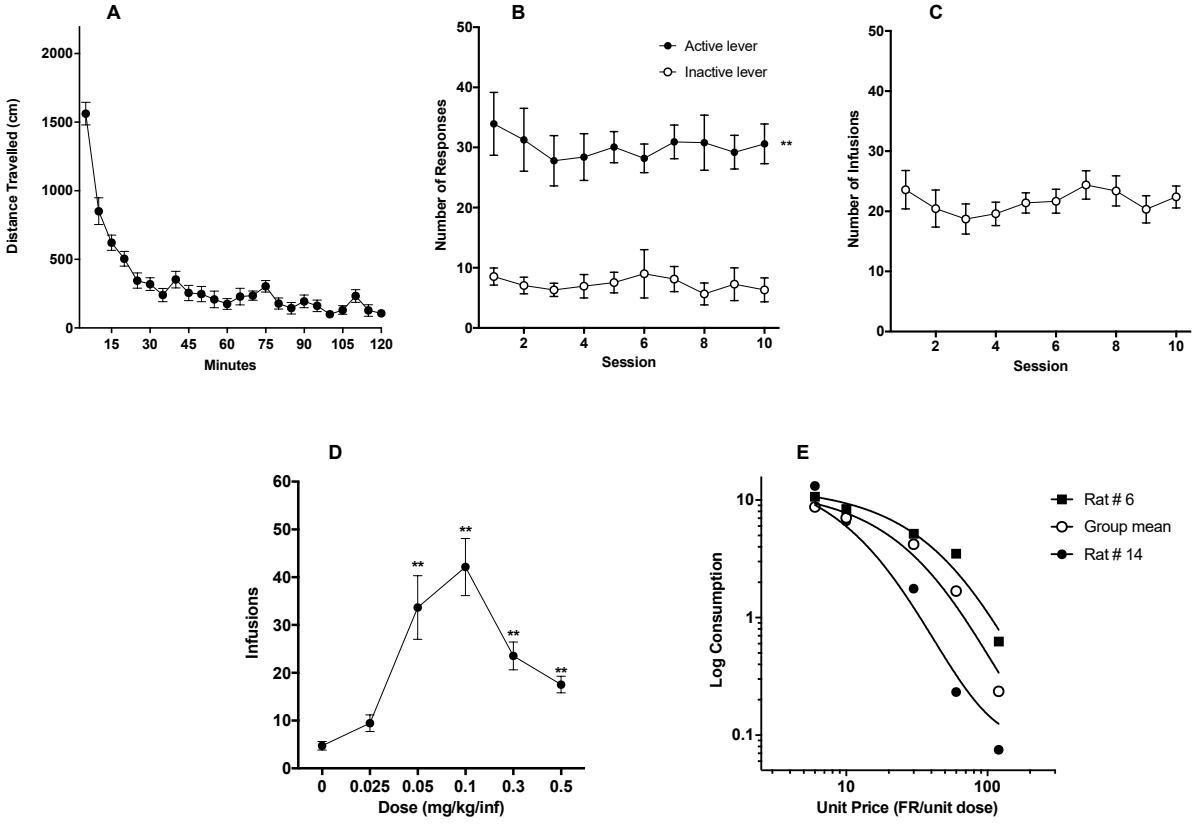


Figure 2. Relationship between distance traveled during the 2 hour locomotor activity test and (A) mean daily infusions during the first 10 sessions of acquisition, (B) mean daily infusions during the first 10 minutes of each session during the first 10 acquisition sessions, (C) number of days to acquire, (D) elasticity of demand (log α) and (E) intensity of demand (Q_0).

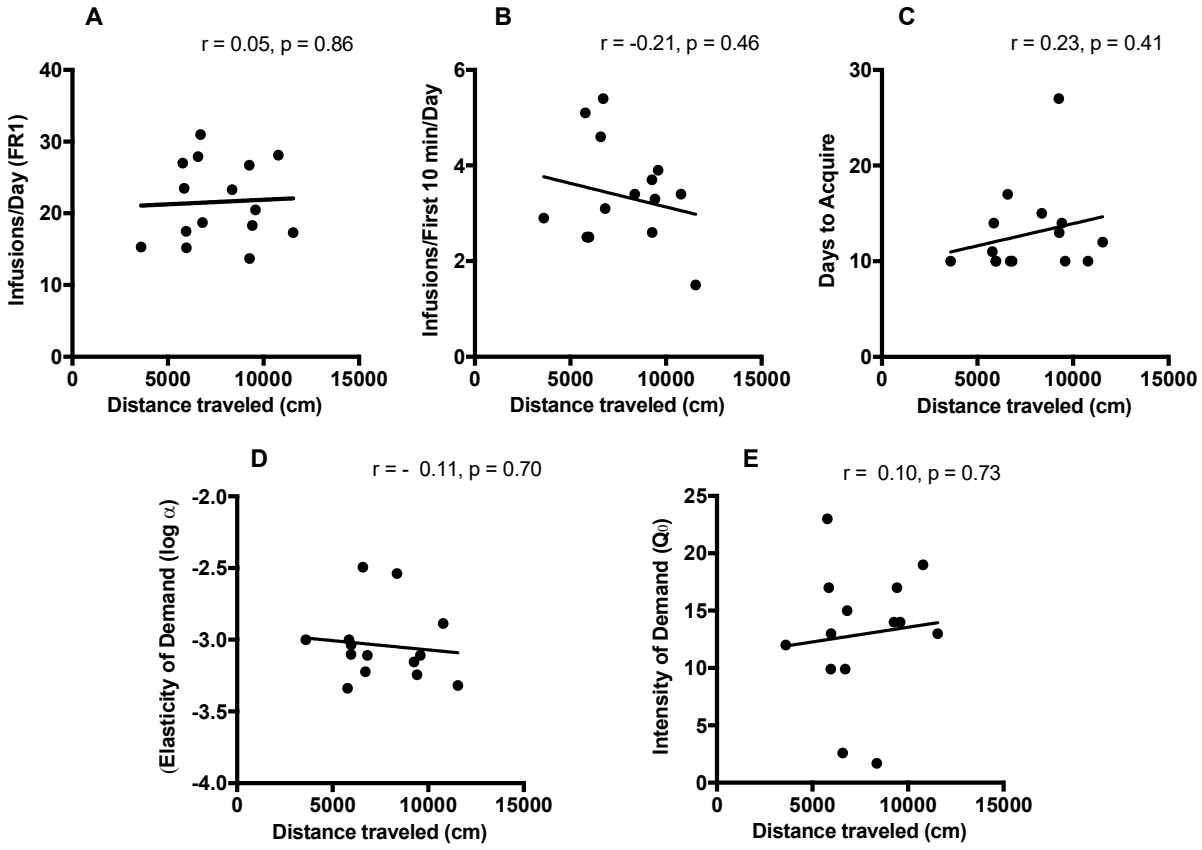


Figure 3. (A) Mean (± SEM) distance traveled in 5-minute blocks during locomotor testing. Mean (± SEM) response on the active and inactive levers (B) and total infusions per session (C) during the first 10 sessions of acquisition in rats responding for morphine (0.2 mg/kg/inf) or saline (0 mg/kg/inf) rats in Experiment 2. (D) Mean (± SEM) number of infusions at FR during demand testing. (E) Exponential demand curve describing morphine consumption as a function of unit price for rats as a group.

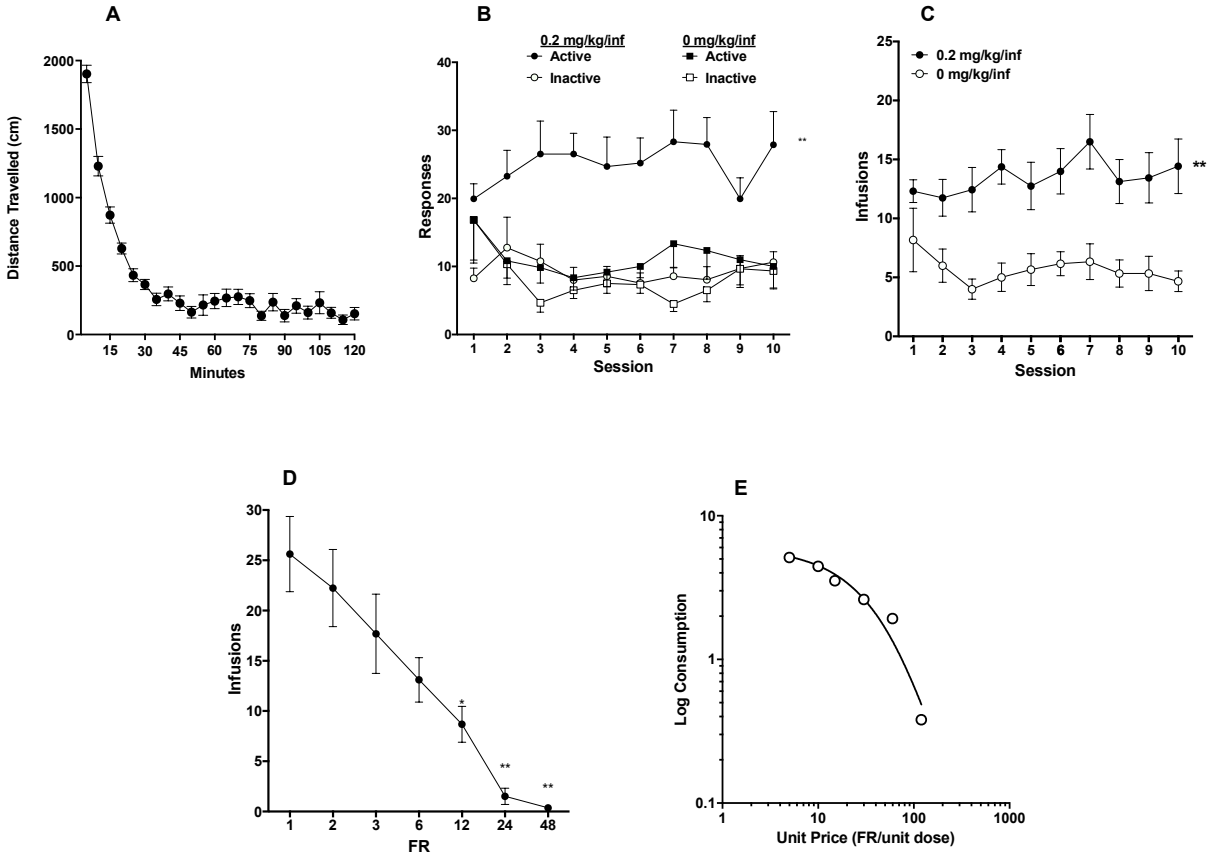
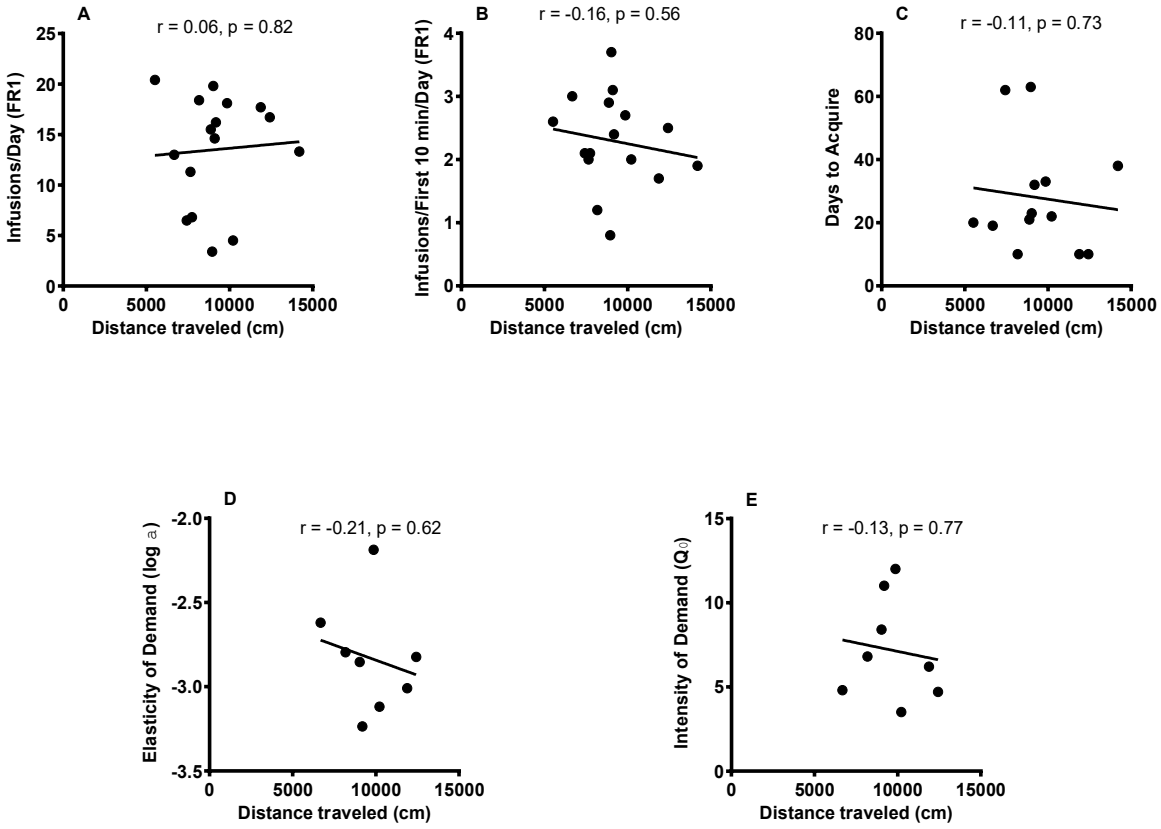


Figure 4. Relationship between distance traveled during the 2 hour locomotor activity test and (A) mean daily infusions during the first 10 sessions of acquisition, (B) mean daily infusions during the first 10 minutes of each session during the first 10 acquisition sessions, (C) number of days to acquire; (D) elasticity of demand (log □), and (E) intensity of demand (Q0) in the morphine SA group. There are fewer data points for (C-E) than in (A) and (B) due to attrition (see text).



Discussion

The main finding of this study is that spontaneous locomotor activity did not predict acquisition of morphine self-administration (SA) in rats using two distinct morphine SA protocols.

To the extent that activity in an open field is relevant to sensation-seeking, our data are consistent with some human studies indicating a lack of positive relationship between sensation seeking and opioid addiction vulnerability (Ahn & Vassileva, 2016; Marino et al., 2013; Nielsen et al., 2012). For example, a recent analysis showed that high sensation-seeking and impulsivity are predictors of greater vulnerability to amphetamine addiction but not heroin addiction (Ahn & Vassileva, 2016). In contrast, other human studies indicate that sensation-seeking is positively associated with opioid addiction vulnerability (Cheng et al., 2015; Franques et al., 2003; Kosten et al., 1994). Taken together, these data suggest that the relationship between sensation-seeking and opioid addiction vulnerability may be complex and may not be observable under all conditions.

Our data with outbred rats contrast with a previous report that inbred rat strains with higher rates of spontaneous locomotor activity also exhibited greater acquisition of morphine SA (Ambrosio et al., 1995). This discrepancy may reflect genetic differences between outbred and inbred rat strains (Zhou et al., 2008) and/or our use of a within-strain rather than between-strain comparison. In addition, the significant between-strain correlation reported in Ambrosio et al. (1995) was only observed when activity was measured *after* catheterization. This relationship was lost when activity was measured *before* catheterization (i.e., catheterization changed the rank order of inbred strains in terms

of locomotor activity), which is the approach used in this study and most others evaluating activity/drug SA relationships. These factors, as well as other methodological differences across studies (e.g., unit dose), could account for the difference.

Based on their findings that spontaneous locomotor activity predicted acquisition of both food and cocaine SA, Mitchell and colleagues suggested that locomotor activity predicts a general learning capability for operant lever pressing rather than drug use propensity *per se* (Mitchell et al., 2005). Our findings that locomotor activity did not predict acquisition of morphine SA contrasts with this prediction, and suggests that the relationship between activity and self-administration may be more complex.

Our current extension of behavioral economics to morphine SA further supports the precision, generalizability, and utility of this analytical approach. An exponential demand function described morphine consumption well, consistent with findings using drugs and other reinforcers in animals and humans (Grebenstein et al., 2013, Hursh, 1991, Hursh & Silberberg, 2008). Importantly, considerable individual differences were observed in α in both studies (Table 3), supporting the use of this approach to measure individual differences in addiction vulnerability. Evaluation of other factors (e.g., opioid withdrawal sensitivity) as determinants of individual differences in demand for morphine is warranted.

The primary goal of this study was to understand the relationship between locomotor activity and morphine SA rather than direct comparison between the determinants of individual differences in morphine versus stimulant addiction. Nonetheless, our data contrast with findings indicating that activity reliably predicts acquisition of SA and reinforcing efficacy of stimulants (e.g., cocaine, amphetamine,

nicotine) (Belin et al., 2011, Belin et al., 2016, Suto et al., 2001; Turner et al., 2008; Piazza et al., 2000). They also complement studies that other predictors of stimulant SA (e.g., impulsivity) do not predict individual differences in opioid SA (Ahn & Vassileva, 2016; Dilleen et al., 2012; McNamara et al., 2010). The cause of these potential differences across drug classes is unclear, but may include differences in their neurobiological effects (e.g. drug-induced synaptic and structural plasticity) or different needs for self-medication (Badiani et al., 2011; Markou et al., 1998). That is, due to the different psychoactive effects of opioids versus stimulants (e.g., anxiolytic effects versus arousal), individuals with varying psychological characteristics (e.g., degree of sensation-seeking) might prefer one drug class over the other (Khantzian, 1985; Markou et al., 1998).

Alternatively, the lack of concordance between the current findings and those with other drugs may reflect methodological factors unique to this study (rat strain, equipment, etc.) rather than our use of an opioid instead of other drugs. However, the rat strain, equipment, and general procedure used here were almost identical to that used in several studies reporting positive relationships between activity and acquisition of SA of non-opioids (e.g., Belin et al., 2008; Smith et al., 2015; Suto et al., 2001). Furthermore, the ability of activity to predict acquisition of SA of other drugs has remained robust despite numerous methodological differences across studies and laboratories including variations in rat strain, method of activity assessment (e.g., circular runway versus open field), operant response (lever press versus nose-poke), etc. Indeed, locomotor activity has been described as the most reliable predictor of acquisition of drug SA in the preclinical individual differences literature (Bardo et al., 2013; Blanchard et al., 2009). Nonetheless,

inclusion of a positive control group tested under the same conditions but responding for a stimulant (e.g., cocaine) in future studies is needed to confirm that our findings reflect a true difference in predictors of SA across drugs. Further evaluating the relationship between activity and opioid SA using other unit doses, durations of access, types of opioids, etc., is also needed to confirm the generality of our findings. Understanding the ability of other putative measures of sensation-seeking (e.g., preference for a novel environment) (Belin et al., 2011; Cain et al., 2005) to predict individual differences in opioid SA is also of interest.

There are several limitations to our study. First, group sizes were relatively small, which may have limited our ability to detect a significant relationship between activity and morphine SA. However, previous studies have detected correlations between locomotor activity and acquisition of stimulant SA using similar or even smaller group sizes (e.g., Mitchell, Cunningham & Mark, 2005; Smith et al., 2015). Given the absence of even a trend for activity to predict acquisition measures in either experiment (see Fig 2 and 4), it is unlikely that the use of larger group sizes would have changed our conclusions regarding these relationships. An additional limitation is that some rats in Experiment 1 exhibited self-mutilation during dose-response testing, which required testing some rats for a shorter number of days at 1-2 of the 6 unit doses. However, removal of data for these animals did not change our conclusions. Importantly, this issue was not present during acquisition, which was our primary outcome.

While not a primary goal of this study, our use of open-field activity testing provided the opportunity to examine the relationship between time in the periphery versus

center of the activity chamber (i.e., thigmotaxis), a measure of anxiety-like behavior (Cohen et al., 2009; Prut & Belzung, 2003; Treit & Fundytus, 1988), and individual differences in morphine SA. Anxiety has been linked to opioid addiction vulnerability in humans (Lejuez et al., 2008; Martins et al., 2012; Norton, 2001), and anxiety-like behavior in the elevated plus maze predicted individual differences in cocaine self-administration in rodents (Dilleen et al., 2012; Pelloux et al., 2009; Walker et al., 2009). We found that thigmotaxis did not predict individual differences in any measure of morphine SA, which is consistent with findings indicating that anxiety-like behavior in the elevated plus maze did not predict individual differences in heroin self-administration (Dilleen et al., 2012).

In conclusion, our findings indicate that locomotor activity in a novel environment did not predict individual differences in morphine SA in rats. These data complement findings from some human studies and suggest that the role of sensation-seeking in individual differences in opioid addiction vulnerability may be limited.

Chapter 3: Higher anhedonia during withdrawal from initial opioid exposure is protective against subsequent opioid self-administration in rats

Opioid addiction poses a substantial burden on public health (Center for Behavioral Health Statistics and Quality, 2018; Jones et al., 2018). Identifying behavioral and neurobiological factors contributing to the marked individual differences in opioid addiction vulnerability is essential for developing more effective preventions and treatments (Belin et al., 2016; Wang et al., 2019). However, few behavioral measures have been identified in preclinical models that reliably predict individual differences in i.v. opioid self-administration (SA). SA is often considered the "gold standard" for modeling addiction-like behavior in animals because it involves volitional drug consumption as occurs in humans (Yap & Miczek, 2008).

Sensitivity to the initial acute effects of drugs (e.g., euphoria, aversion) is a key predictor of addiction vulnerability in humans (O'Loughlin et al., 2003; DiFranza et al., 2007; Schuckit et al., 2004) and the direction of the relationship depends on the drug effects measured. For example, greater aversive effects of initial drug exposure can be protective against the subsequent development of addiction (DiFranza et al., 2004; Fowler & Kenny, 2014; Sartor et al., 2010). Similarly, several preclinical studies indicate that sensitivity to the initial effects of acute drug injections (e.g., locomotor activity or depression, antinociception) predict voluntary drug intake in an i.v. SA model (Deminiere et al., 1989; Chappell & Weiner, 2008; Nishida et al., 2016).

Acute drug injections also produce negative affective (emotional) states (e.g., anhedonia, or diminished reward sensitivity) during withdrawal. These withdrawal effects

are induced even after a single drug exposure in both humans and animals ("acute dependence"; Harris & Gewirtz, 2004; Liu & Schulteis, 2004; Schulteis et al., 2004; Harris & Gewirtz, 2005), and often become more severe with repeated drug exposures (Engelmann et al., 2004; Harris et al., 2004; Kenny et al., 2003). Some authors have proposed that greater sensitivity to the negative affective consequences of withdrawal may be protective against addiction (Carroll et al., 2008; Dess et al., 2005; Holtz et al., 2015; O'Dell, 2009; O'Dell et al., 2006), and that anhedonia may reduce the motivation for reward-seeking (Wise, 2004). Consistent with these predictions, rats selectively bred for high voluntary alcohol consumption showed lower withdrawal-induced anhedonia (WIA) after initial alcohol exposure (Chester et al., 2006). Similarly, it was found that saccharin-preferring rats, which exhibit greater SA of opioids and other drugs (Carroll et al., 2002), exhibit lower WIA during withdrawal from acute morphine exposure (Holtz et al., 2015). Nevertheless, the relationship between sensitivity to withdrawal from acute drug exposure and drug SA has not been directly tested within the same animals or in outbred rats, which can differ from selectively bred rats in terms of determinants of addiction vulnerability (Ambrosio et al., 1995; Zhou et al., 2008).

The goal of this study was to evaluate the ability of WIA to predict individual differences in subsequent i.v. morphine SA (MSA) in outbred rats. WIA was measured as increases in intracranial self-stimulation (ICSS) thresholds, which is one of the most commonly used measures of the anhedonic consequences of withdrawal from opioids and other drugs in rats and has considerable predictive validity (Bruijnzeel et al., 2007; Cahill et al., 2009; Igari et al., 2014).ICSS was also used in the studies discussed above

demonstrating reduced WIA in rodents selectively bred for greater addiction vulnerability (Chester et al., 2006; Holtz et al., 2015) Several common measures of MSA (e.g., acquisition, demand, reinstatement) were used because they model distinct aspects of addiction and can be differentially associated with other behavioral predictors of drug SA (Belin et al., 2011; Belin et al., 2008). It was hypothesized that greater WIA severity would be associated with lower MSA vulnerability.

Materials and Methods

Overview of experimental protocol

Male adult Sprague-Dawley rats were tested under the experimental protocol shown in Figure 5. Rats were first tested for WIA during naloxone-precipitated and spontaneous withdrawal from acute morphine injections (Phase 1). Rats were then tested for opioid addiction vulnerability using several measures of i.v. MSA including acquisition, elasticity of demand, and reinstatement (Phase 2). Finally, WIA was again tested to provide a *preliminary* characterization of the relationship between MSA and withdrawal sensitivity during a more advanced stage of dependence ("late-stage dependence", Phase 3).

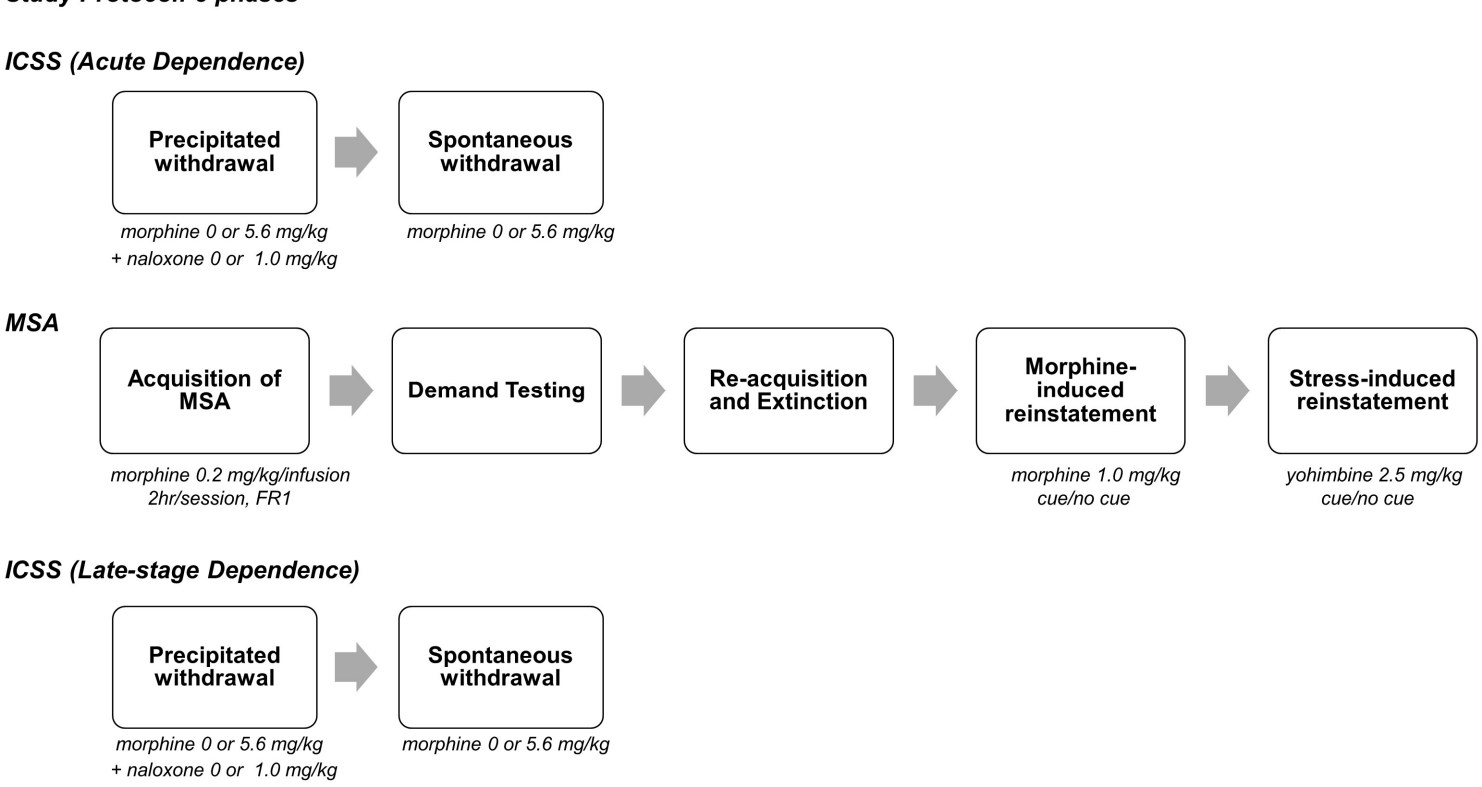
Acute dependence

Rats were prepared and trained on a discrete-trial ICSS procedure (see Supplemental Material) in daily sessions conducted Mon-Fri until ICSS thresholds were stable (<10% variability over 5 days) and habituated to saline injections as described previously (see Harris et al., 2013). On the first test day, rats were injected with morphine sulfate (MOR, 0 or 5.6 mg/kg, s.c., expressed as the salt). One hour and fifty minutes later,

rats were injected with the opioid antagonist naloxone (NX, 0 or 1.0 mg/kg, s.c.) and tested for ICSS 10 minutes later. These morphine and naloxone doses and this pretreatment interval produce significant negative affective morphine withdrawal signs, including WIA (Schulteis et al., 2004; Harris et al., 2004; Holtz et al., 2015). Immediately after ICSS testing, somatic withdrawal signs were assessed as a secondary withdrawal measure (see Supplementary Material). There was a total of 4 groups in a 2 (MOR dose) x 2 (NX dose) factorial design. The MOR + NX group (n = 29) was larger than the MOR + SAL, SAL + NX, and SAL + SAL groups (n = 10-11/group) in order to have adequate power for correlation analysis (see below). These procedures were repeated each day for 5 consecutive days. Animals were then tested for ICSS under drug-free conditions for at least one week and until ICSS thresholds were stable (same stability criteria as above).

To test spontaneous withdrawal, the same rats received a single injection of 5.6 mg/kg MOR (MOR+NX and MOR+SAL groups) or 0 mg/kg MOR (SAL+NX and SAL+SAL groups) and ICSS was tested 2, 6, 26, 30, 50, 64, 74, 98, and 170 hours later. The purpose of the 2 hour time point was to detect any reinforcement-facilitating (ICSS threshold-lowering) effects of morphine itself (see Altarifi & Negus, 2011). The selection of subsequent time points was based on the time course of spontaneous withdrawal from acute morphine exposure determined using ICSS and other measures (Harris & Gewirtz, 2004; Rothwell et al., 2010; Liu & Schulteis, 2004). Somatic withdrawal signs were recorded immediately after ICSS testing at the 26 hour time point (based on Allahverdiyev et al., 2015). ICSS testing was suspended after the 170 hour time point.

Figure 5. Overview of experimental protocol. During the acute dependence phase (Phase 1), precipitated withdrawal was tested repeatedly over 5 consecutive days. On each day, rats were injected with morphine (0 or 5.6, mg., s.c.), followed 1 hr 50 min later by naloxone (0 or 1.0 mg/kg), and then tested for ICSS 10 min later (length of ICSS session ≈ 45 min). After precipitated withdrawal, rats were injected with morphine (0 or 5.6 mg/kg) and tested for ICSS 2, 6, 26, 30, 50, 64, 74, 98, and 170 hours later (Phase 2). After completion of spontaneous withdrawal testing, all animals were tested using various measures of MSA (e.g., acquisition, demand, reinstatement) in daily 2 hr sessions. Following completion of the MSA protocol, rats were again tested for precipitated and spontaneous withdrawal as described for the acute dependence phase (late-stage dependence, Phase 3).



Locomotor activity

Within 48 hours after the final ICSS test, locomotor activity in a novel environment (i.e., "sensation-seeking" (Blanchard et al., 2009; Pawlak et al., 2008; Piazza et al., 1989)) was tested for 2 hours as a secondary predictor of MSA.

MSA

Approximately 24-48 hours after completion of the locomotor activity test, animals from all groups were implanted with i.v. catheters using our standard procedures (see Study 1). Following a 7-10 day recovery period, all rats were allowed to acquire i.v. morphine SA (0.2 mg/kg/inf) during daily 2 hr sessions conducted Mon-Fri using our standard apparatus and procedures (see Supplementary Material). Rats were tested under a fixed ratio (FR) 1 schedule for at least 10 sessions and until acquisition criteria were met (≥5 infusions per session, ≤ 20% coefficient of variation, and ≥ 2:1 response ratio on the active lever to inactive lever) across 3 sessions. To test elasticity of demand (reinforcing efficacy), the FR requirement was increased every 3-4 sessions as follows: FR 2, 3, 6, 12, 24, and doubled thereafter until infusion rates during the last 2 sessions at a given FR were reduced by 90% compared to baseline (FR 1). Morphine consumption under this protocol is well described by the current exponential demand function (Hursh & Silberberg, 2008). Data from Mondays were excluded from data analysis to avoid potential spontaneous recovery of responding after the weekend. Therefore, if one of the three sessions at a given FR occurred on a Monday, rats were tested in an additional session at that FR.

After completion of demand testing, rats were allowed to reacquire MSA under an FR1 schedule for at least 5 sessions and until MSA was stable (same stability criteria as

above). Extinction conditions were subsequently introduced in which the morphine dose was replaced with saline and the drug-associated cue light was no longer presented upon infusion. Extinction was tested for at least 10 sessions and until animals exhibited a 75% reduction in active lever pressing for 2 consecutive sessions.

To test morphine- and cue-induced reinstatement, rats were injected s.c. on separate days with either saline or morphine (1.0 mg/kg) 10 min prior to the SA session. This morphine dose and pretreatment interval reliably reinstated drug-seeking in our lab (data not shown) and others (Vassoler et al., 2017). Responses on the active lever resulted in a saline infusion and either presentation of the drug-associated cue light ("cue" condition) or no programmed consequences ("no cue" condition). Reinstatement testing was therefore conducted using a 2 (morphine dose) x 2 (cue condition) design, resulting in a total of 4 reinstatement tests. These tests were conducted on Tuesdays and Fridays, provided that active lever pressing returned to extinction levels during the preceding session, and the order of reinstatement conditions was counterbalanced. Following completion of morphine- and cue-induced reinstatement testing, rats were tested under extinction conditions for at least 5 sessions and until extinction criteria were again met. Similar within-subject designs are commonly used to study reinstatement (Le et al., 1998; Liu & Weiss, 2002). To test stress- and cue-induced reinstatement, the above procedure was repeated except that rats were injected i.p. with either deionized water or the stress-inducing α2-adrenergic antagonist yohimbine (2.5 mg/kg) 30 min prior to each SA test. This dose of yohimbine and pretreatment interval reliably reinstate extinguished SA (Shepard et al., 2004).

Late-stage dependence

Following completion of all MSA procedures, rats were again tested for ICSS until thresholds were stable. Rats (N = 26) were subsequently tested for precipitated (MOR + NX: n = 14, n = 4/group for other groups) and spontaneous (MOR: n = 15, SAL: n = 5) morphine withdrawal using the same protocol as described for acute dependence testing above. The small group sizes for this exploratory phase reflect the considerable attrition rate by this stage of the protocol (see Supplemental Materials).

Statistical analysis

ICSS

ICSS thresholds (a measure of brain reinforcement function) and response latencies (a measure of non-specific motoric effects; Markou & Koob, 1992) during naloxone-precipitated and spontaneous withdrawal were measured as percentage of baseline (average of the last 5 days prior to onset of withdrawal testing). To provide a composite measure of naloxone-precipitated withdrawal severity for each animal, percentage scores were standardized into z-scores and then averaged across average and peak ICSS thresholds during all 5 precipitated withdrawal tests and degree of sensitization of WIA (the difference score in ICSS thresholds between test days 1 and 5) with the following formula:

$$\frac{Z(\text{average thresholds}) + Z(\text{peak thresholds}) + Z(\text{difference score of thresholds})}{3}$$

Spontaneous withdrawal severity was measured as peak withdrawal severity during hours 6 – 98 after morphine injection. This measure accounts for the considerable individual differences in the time course of changes in ICSS thresholds during spontaneous withdrawal (see Harris et al., 2011). In the few cases (n = 2 for precipitated withdrawal

during 1 or 3 sessions and n = 1 for spontaneous withdrawal during 1 session) where rats failed to respond for any ICSS current intensity, we arbitrarily assigned ICSS threshold and latency values based on those obtained in the animal achieving the highest ICSS threshold in that phase of the experiment (see Harris et al., 2015; Markou & Koob, 1991).

MSA

MSA acquisition was measured as the mean number of infusions per session during the first 10 days of acquisition. To determine opioid reinforcing efficacy during FR escalation, exponential demand curve analyses were conducted as described in detail elsewhere Hursh & Silberberg, 2008). Consistent with previous studies (Diergaarde et al., 2008; Hursh & Silberberg, 2008; Study 1), we used α as our primary demand measure. This outcome refers to the rate of change in consumption with increases in unit price (elasticity of demand), with higher α values indicating lower reinforcement efficacy. Zero values in consumption were replaced with 0.01 ($1/10^{th}$ of our lowest non-zero consumption level) to provide better curve fits and more accurate parameter estimates of demand for individual rats (Koffamus et al., 2015; Murphy et al., 2009). α values were log-transformed due to non-normal distribution. Extinction was measured as mean number of infusions per session during the first 10 sessions of extinction. Degree of reinstatement (the reinstatement score) was defined as the difference between active and inactive lever responses during each reinstatement test (Cippitelli et al., 2010; Le et al., 2005; Tran-Nguyen et al., 1998).

All statistical analyses and graphing were performed in GraphPad Prism 7 or R 3.4.3, with significance level set at $\alpha = 0.05$ for all tests. In general, data were analyzed

using ANOVA followed by Holm-Sidak's or Dunnet's multiple comparison tests (see Results for more details). Relationships between ICSS and MSA measures in the MOR + NX group were assessed using Pearson's correlation.

Results

Acute dependence

Precipitated withdrawal: ICSS

Baseline ICSS thresholds did not differ between groups (Table S1). A two-way ANOVA on ICSS thresholds during precipitated withdrawal revealed a significant main effect of group ($F(3, 54) = 36.62$, $p < 0.0001$) and a significant interaction between group and session ($F(12, 216) = 3.181$, $p = 0.0003$). Dunnett's multiple comparisons indicated that ICSS thresholds were significantly elevated in the MOR+NX group compared to SAL+SAL controls during all 5 sessions (all $q \geq 3.162$, all $p \leq 0.005$). In contrast, thresholds in the MOR+SAL and SAL+NX groups did not differ from the SAL + SAL group during any session (Figure 6A). A repeated-measures ANOVA showed a significant effect of session in the MOR+NX rats ($F(2.863, 77.30) = 18.40$, $p < 0.001$), with Dunnett's multiple comparisons indicating significantly higher ICSS thresholds during sessions 2-5 compared to session 1 (all $q \geq 4.68$, all $p \leq 0.001$). In contrast, there was no effect of session in any of the control groups (all $p > 0.05$).

No significant differences were observed in baseline ICSS response latencies between groups (Table S1). Latencies also did not differ between groups during precipitated withdrawal testing (Figure 6B), indicating the absence of non-specific (e.g., motoric) effects.

Spontaneous withdrawal: ICSS

Baseline ICSS thresholds did not differ between groups (Table S1). ICSS threshold data during hours 2 (i.e., acute effect of MOR itself) and hours 6 – 98 (i.e., withdrawal period) of spontaneous withdrawal did not differ between the two groups receiving MOR (MOR + NX and MOR + SAL groups) or between the two groups receiving SAL (SAL + SAL and SAL + NX groups). Therefore, data from these groups were combined into single MOR (n = 37) and SAL (n = 20) groups for further analysis. Welch's corrected t-test showed no significant difference in ICSS thresholds between MOR and SAL rats 2 hours after injection (MOR: 107.7 ±5.35%; SAL: 100.4 ± 2.50%), indicating that MOR itself did not affect ICSS. Two-way ANOVA on ICSS thresholds during spontaneous withdrawal 6-98 hours after morphine injection revealed a significant main effect of time ($F(7, 385) = 4.831$, $p < 0.0001$) and group (morphine vs. saline) ($F(1, 55) = 4.012$, $p = 0.05$), but no significant interaction (Figure 6C). After correcting for multiple comparisons, ICSS did not significantly differ between groups at any individual time-point post injection. However, peak ICSS threshold values between hours 6 and 98 (regardless of the time point at which they occurred) differed significantly between the morphine (117.1 ± 2.15%) and saline (110.4 ± 1.63%) groups (Welch-corrected $t(54.9) = 2.50$, $p = 0.02$).

No significant differences were observed in ICSS response latencies between groups during baseline sessions (Table S1), 2 hours after injection (MOR: 103.2 ± 3.01%; SAL: 96.43 ± 1.82%), or during spontaneous withdrawal (Figure 6D).

Precipitated withdrawal: Somatic signs

One-way ANOVA on total somatic signs during the 5th session of naloxone-precipitated withdrawal indicated a significant effect of group ($F(3, 52) = 11.51$, $p < 0.0001$). Dunnett's multiple comparison test revealed significantly higher scores in the MOR + NX group compared to the SAL + SAL group ($q(52) = 2.62$, adjusted $p = 0.03$). In contrast, neither the MOR + SAL or SAL + NX group differed significantly from the SAL + SAL group (Figure 7A).

Spontaneous withdrawal: Somatic signs

During spontaneous withdrawal, somatic sign scores did not differ between the two groups receiving MOR (MOR + NX and MOR + SAL groups) or between the two groups receiving SAL (SAL + SAL and SAL + NX groups). Data from these groups were therefore combined into a single MOR ($n = 37$) or SAL ($n = 20$) condition. T-tests showed that scores were significantly higher in the MOR condition compared to the SAL condition (Welch-corrected $t(51.31) = 2.25$, $p = 0.03$) (Figure 7B). Eye blinking, facial fasciculations and swallowing movements were the most commonly observed somatic signs during both precipitated and spontaneous withdrawal (Table S4, S5).

MSA in the Mor + NX Group

Acquisition (n = 29)

Two-way ANOVA revealed a significant main effect of lever (active vs inactive) ($F(1, 28) = 58.38$, $p < 0.0001$) and session ($F(9, 252) = 2.896$, $p = 0.003$) on responses during the first 10 days of acquisition (Figure 8A). Sidak's multiple comparison test showed significantly higher responses on the active lever during all acquisition sessions (all $t \geq 4.88$, all $p < 0.0001$).

Demand (n = 25 due to attrition, see Supplementary Material)

Increases in FR requirement resulted in a progressive reduction in morphine consumption (Figure 8B). A one-way repeated measures ANOVA revealed a significant effect of FR on number of morphine infusions ($F (3.59, 82.63) = 79.43$, $p < 0.0001$). A post-hoc Dunnett's multiple comparisons test showed that infusions at all subsequent FRs were significantly lower than at FR 1 (all $q \geq 4.23$, all $p \leq 0.002$). Morphine consumption during demand testing was well-described by an exponential demand function, with R^2 values typically ≥ 0.80 for individual animals (Table S2) and $R^2 = 0.94$ for rats as a group, with considerable individual variability in elasticity of demand (see Figure 8C).

Extinction (n = 24)

Repeated-measures ANOVA revealed a significant main effect of session ($F (10, 230) = 27.14$, $p < 0.001$), main effect of lever ($F (1, 23) = 67.44$, $p < 0.001$; Figure 8D), and an interaction between session and lever ($F (10, 229) = 26.98$, $p < 0.001$) during extinction. A post-hoc Holm-Sidak's multiple comparisons test showed that active lever presses were significantly higher than inactive lever presses during sessions 1-7 (all $p < 0.05$), but not during sessions 8-10.

Morphine-Induced Reinstatement (n = 23)

One-way repeated measures ANOVA of reinstatement scores (responses on the active lever - inactive lever) during morphine-induced reinstatement showed an overall effect of treatment ($F(1.12, 24.73) = 18.18$, $p < 0.001$) (Figure 9A). Holm-Sidak's multiple comparisons test revealed that the MOR + NO CUE, VEH + CUE and MOR + CUE conditions all resulted in significantly higher responding compared to the VEH + NO CUE

condition (all $t \geq 4.82$, all $p < 0.001$). MOR+CUE resulted in significantly higher responding than VEH + CUE ($t = 3.83$, $p = 0.002$) and MOR + NO CUE ($t = 3.80$, $p = 0.002$).

Stress-Induced Reinstatement (n = 22)

There was an overall effect of treatment on reinstatement scores during stress-induced reinstatement ($F(1.68, 35.35) = 8.92$, $p = 0.001$) (Figure 9B). Holm-Sidak's multiple comparisons test revealed that VEH + CUE ($t = 5.98$, $p < 0.001$) and YOH + CUE treatment ($t = 3.75$, $p = 0.006$) resulted in higher responding than the VEH + NO CUE control condition, whereas the YOH + NO CUE condition did not. Responding during YOH + CUE reinstatement was significantly higher than during the YOH + NO CUE condition ($t = 3.17$, $p = 0.02$), but did not differ from the VEH + CUE condition.

Correlations in the MOR + NX group

Composite z-scores for ICSS thresholds during precipitated withdrawal were significantly correlated with all individual measures: peak ICSS threshold ($r = 0.92$, $p < 0.001$), average ICSS threshold ($r = 0.86$, $p < 0.001$) and degree of sensitization of WIA ($r = 0.54$, $p = 0.003$). This validates our use of z-scores to measure cumulative precipitated withdrawal severity (see Belin et al., 2009; Belin & Deroche-Gamonet, 2012). Pearson's r revealed that greater composite WIA severity during precipitated withdrawal correlated with lower infusions during acquisition of MSA and higher elasticity of demand (i.e., lower reinforcing efficacy) (Table 4, Figure 10). Greater peak ICSS threshold elevation during spontaneous withdrawal was associated with lower acquisition and reinstatement induced by morphine alone or morphine + cue (Table 4, Figure 11), and marginally correlated with

elasticity of demand ($p = 0.06$, Figure 11). Most MSA measure correlated significantly with at least one other MSA measure (Table 4). However, no individual MSA measure correlated with as wide a range of other MSA measures as did WIA (Table 4).

Figure 6. Mean (± SEM) ICSS thresholds (A) and response latencies (B) (expressed as percent of baseline) during naloxone-precipitated withdrawal (acute dependence). Mean (± SEM) ICSS thresholds (C) and latencies (D) as percent of baseline during spontaneous withdrawal (acute dependence). *,** Different from SAL+SAL group at that session or SAL condition during hours 6-98 (main effect), $p < 0.05, 0.01$. # Different from Session 1 in that group, $p < 0.05$.

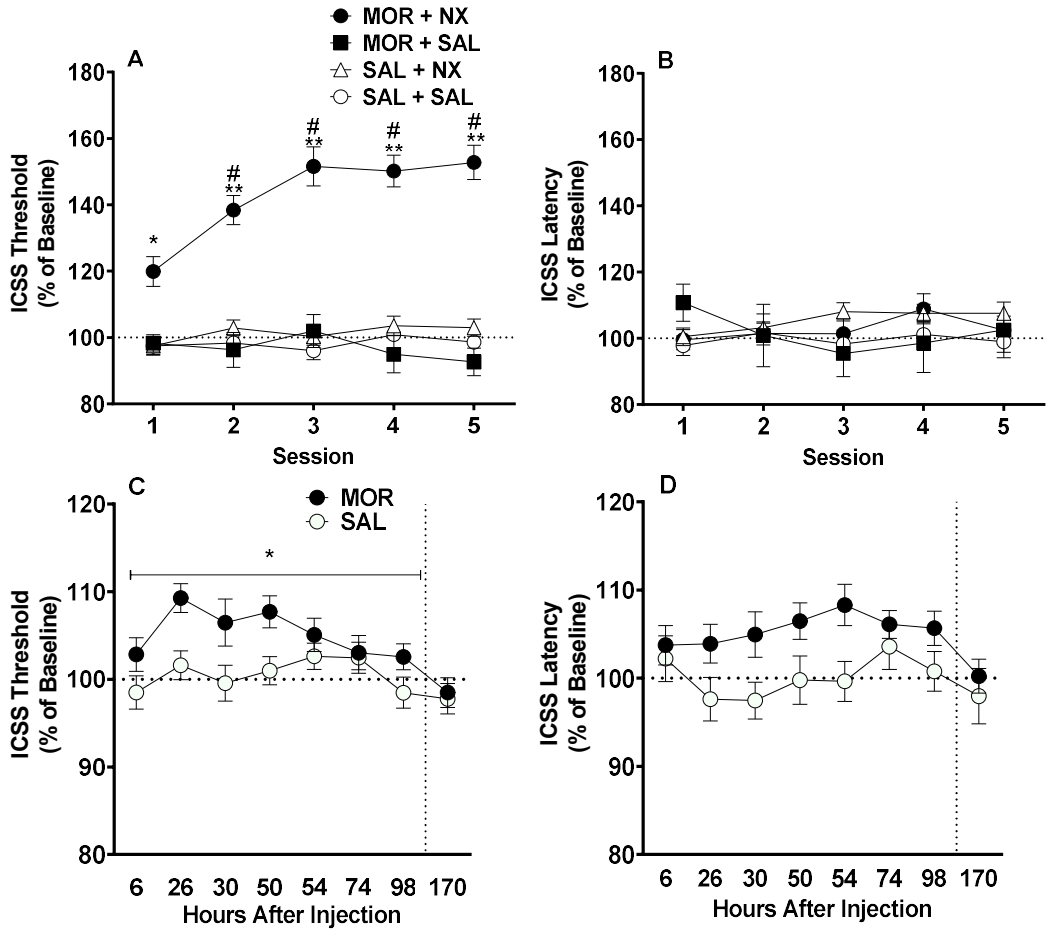


Figure 7: (A) Mean (± SEM) somatic signs in groups on the 5th day of acute dependence precipitated withdrawal testing. (B) Mean (± SEM) somatic signs in the MOR and SAL condition 26 hours after injection during acute dependence spontaneous withdrawal testing. *** Significant effect of group, $p < 0.001$. * Different from SAL + SAL group or SAL condition, $p < 0.05$.

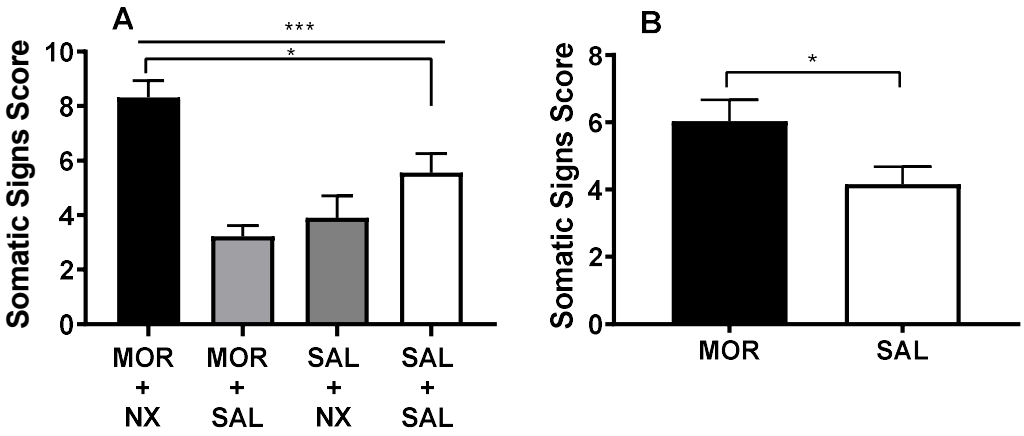


Figure 8. MSA in MOR + NX rats. (A) Mean (± SEM) active and inactive lever presses and infusions during the first 10 sessions of acquisition. ** Different between active and inactive lever presses, $p < 0.01$. (B) Mean (± SEM) infusions at each FR during demand testing. ** Different compared to infusions at FR1, $p < 0.01$. (C) Exponential demand curve describing morphine consumption as a function of unit price for rats as a group, and for individual rats with relatively high (rat #26) and low (rat #9) elasticity of demand (α). (D) Mean (± SEM) infusions during baseline and during the first 10 extinction sessions, ** Different compared to pre-extinction (baseline), $p < 0.01$. The increase in infusion rates during session 6 reflects spontaneous recovery following the weekend break in extinction testing.

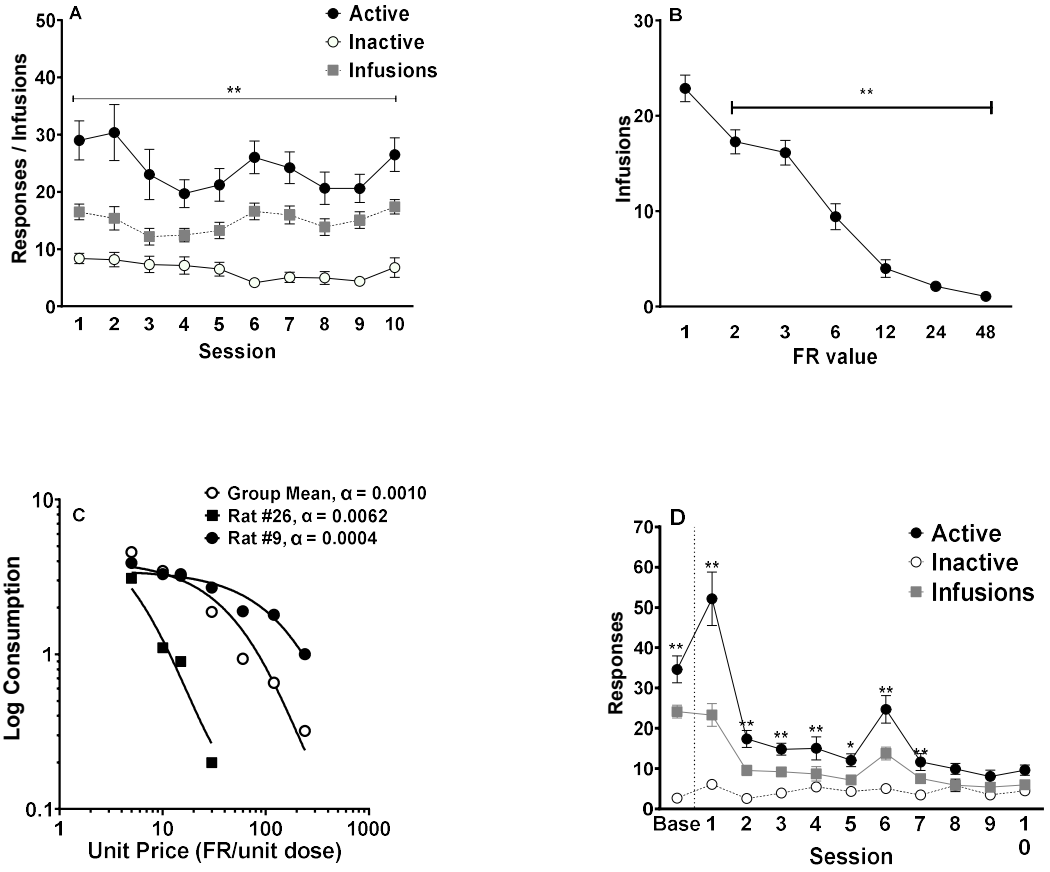


Figure 9. Mean (± SEM) reinstatement scores (differences between active and inactive lever responses) during morphine- and cue-induced reinstatement (E) and yohimbine-and cue-induced reinstatement (F). Active and inactive lever presses during each reinstatement test are shown in the insets. ** Different compared to VEH + NO CUE, $p < 0.01$.

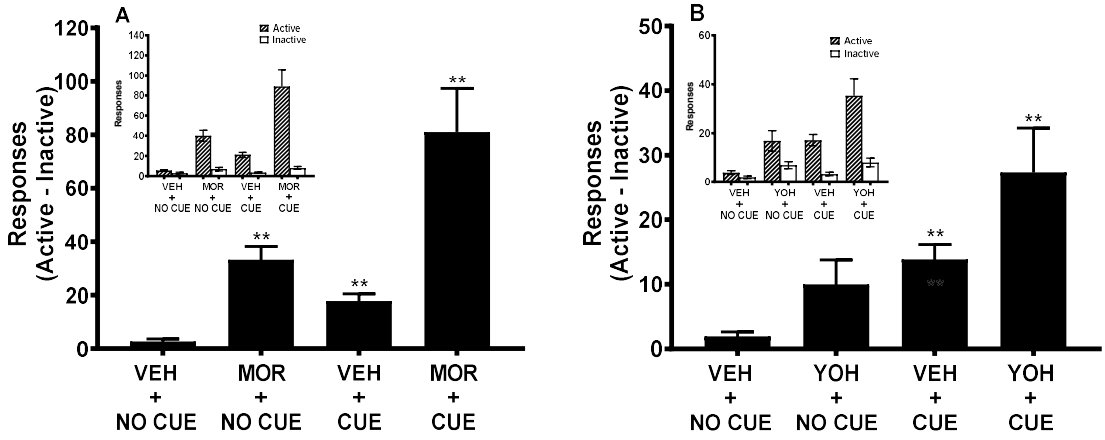


Figure 10: Scatterplots with regression line depicting the relationship between naloxone-precipitated withdrawal measured using ICSS (composite z-score) and infusions during first 10 sessions of acquisition (A), log α (B), infusions during first 10 sessions of extinction (C), and reinstatement score during MOR + CUE (D), MOR + NO CUE (E) and YOH + CUE (F) reinstatement. Higher log α (elasticity of demand) = lower reinforcement efficacy.

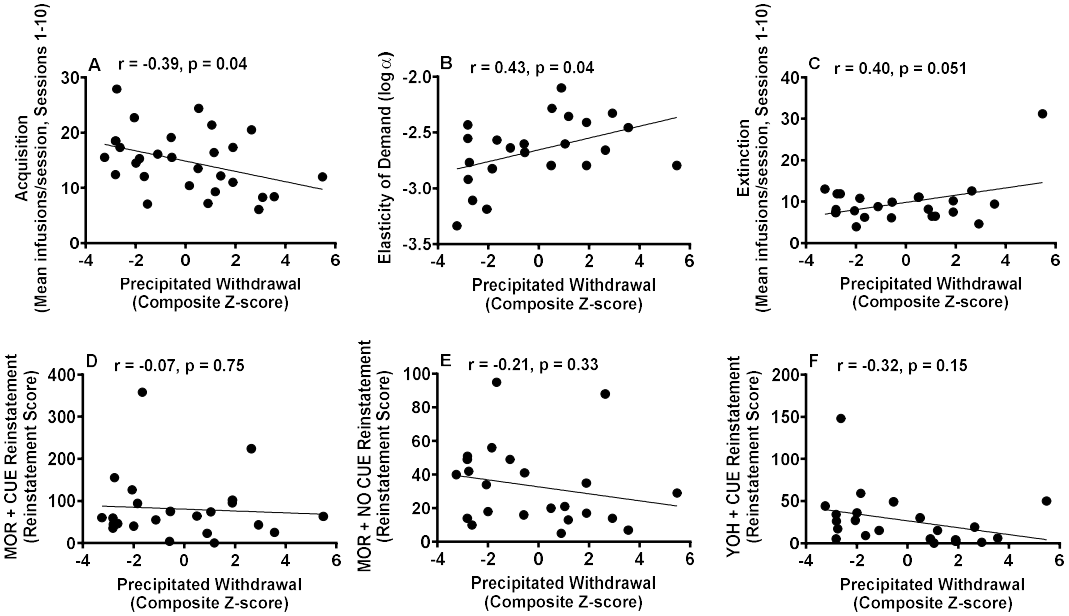
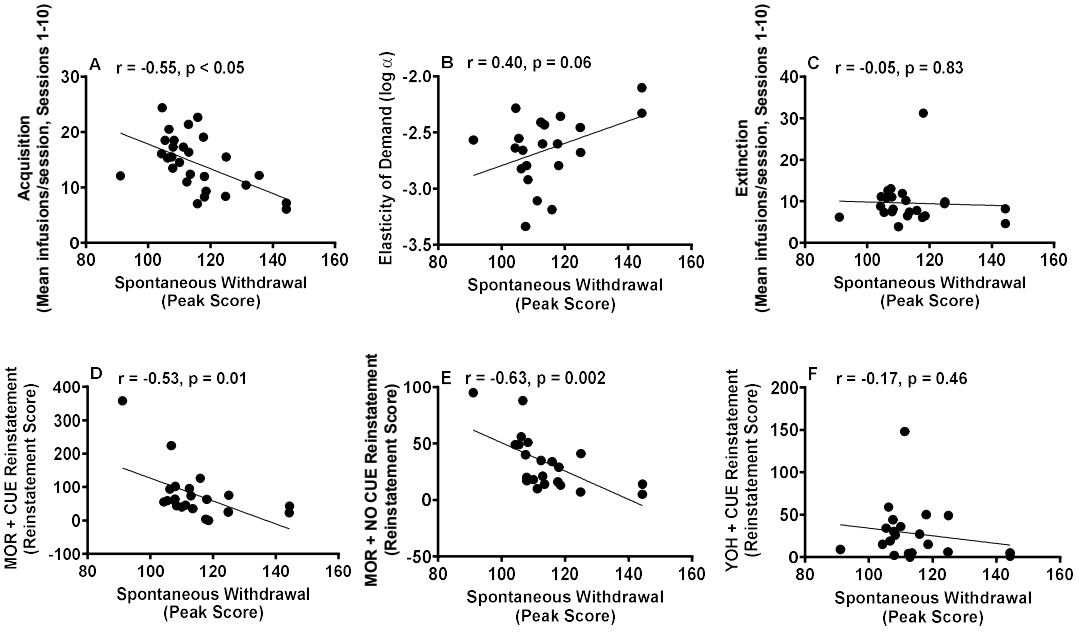


Figure 11: Scatterplots with regression line depicting the relationship between peak spontaneous withdrawal during ICSS testing and infusions during first 10 sessions of acquisition (A), log α (B), infusions during first 10 sessions of extinction (C), and reinstatement score during MOR + CUE (D), MOR + NO CUE (E) and YOH + CUE (F) reinstatement in the Mor + NX group. Higher log α (elasticity of demand) = lower reinforcement efficacy.



Additional Analyses

Secondary correlations in the MOR + NX group

There were no significant correlations between any of the secondary predictors (i.e., somatic signs during acute dependence testing, locomotor activity) and any measure of MSA (all p-values > 0.05, data not shown).

Locomotor activity and MSA in control groups

Rats in the control groups did not differ from the MOR + NX group in terms of locomotor activity or on any primary MSA measure (see Supplementary Materials, Figure S1A, S2).

Late-stage dependence

ICSS thresholds were significantly elevated during precipitated withdrawal during late-stage dependence (Figure S3), while somatic signs were significantly elevated during spontaneous withdrawal (Figure S3). However, these effects were not correlated with most MSA measures (see Supplementary Material).

Attrition

Several animals (see Results for group size of the MOR + NX group during each phase of the MSA protocol) were lost to attrition during the course of the protocol due to loss of ICSS headcap, loss of stability of ICSS thresholds, failure to acquire MSA, loss of catheter patency, health issues, or other problem. Data for these animals are analyzed for those phases they completed.

Baseline ICSS measures during acute dependence

Baseline ICSS thresholds and response latencies did not differ between groups during either precipitated or spontaneous withdrawal testing during the acute dependence phase (Table S1).

Locomotor activity

One-way ANOVA on total distance travelled during the locomotor activity session in the MOR + NX and control groups (MOR + SAL, SAL + NX, SAL + SAL) indicated no effect of group (Fig S1A). An additional one-way ANOVA of within-session activity in the MOR + NX group indicated a main effect of time ($F(3.417, 95.66) = 64.07$, $p < 0.0001$). Activity levels were highest during the first 30 minutes of the 2-hour session (Fig S1B), consistent with previous findings from our lab and others (e.g., Study 1, Piazza et

al., 1989). Distance travelled did not correlate with any subsequent MSA measure (all $p \geq 0.11$).

Comparison of MSA in the MOR + NX and control groups

Rats in the MOR + NX group and control groups did not differ on most measures of MSA. Two-way repeated measures ANOVA on infusion rates between the MOR + NX and the control groups during the first 10 days of acquisition indicated a significant overall effect of session ($F (9, 468) = 2.20$, $p = 0.02$) but no effect of group or interaction between group and session (Figure S2A). Two-way ANOVA on group and FR during demand testing showed a significant main effect of FR ($F (6, 246) = 152$, $p < 0.001$) and interaction between group and FR ($F (18, 246) = 2.144$, $p = 0.005$). but no main effect of group (Figure S2B). The MOR + NX had significantly lower infusions at FR 2 and FR 3 compared to the SAL + SAL group (all $p < 0.01$). However, a one-way ANOVA comparing elasticity of demand (α) (i.e., the primary outcome) indicated no significant difference between groups (Figure S2C). The MOR + NX and control groups also did not significantly differ in three secondary behavioral economic measures: intensity of demand (Q_0), maximal response output (O_{max}) and the unit price at which maximal response output occurred (P_{max}) (Table S2). Additionally, the groups did not differ in infusions during extinction, as a two-way repeated measures ANOVA revealed a significant overall effect of session ($F (10, 410) = 79.53$, $p < 0.0001$) (Figure S2D) but no effect of group or group x session interaction. Finally, two-way ANOVAs showed a main effect of reinstatement condition on reinstatement scores during morphine- and cue-induced reinstatement ($F (1.291, 51.65) = 28.95$, $P < 0.0001$) and yohimbine- and cue-induced reinstatement ($F (1.747, 65.79) =$

22.68, $P < 0.0001$), with responses significantly elevated during all drug (morphine or yohimbine) and/or cue conditions compared to the VEH + CUE control condition (Figure S2E-F). However, there was no significant main effect of group or interaction during either morphine- and cue-induced reinstatement testing or yohimbine- and cue-reinstatement testing.

Additional behavioral economics measures

Table S2 shows additional parameters from the exponential demand curve, However, these additional measures (Q_0, O_{max} or P_{max}) from demand testing did not significantly correlate with either ICSS thresholds or somatic withdrawal signs during naloxone-precipitated or spontaneous withdrawal in the Mor + NX group during the acute dependence phase.

Late-stage dependence

Precipitated withdrawal: ICSS

Baseline ICSS thresholds and response latencies for late-stage precipitated withdrawal did not differ between groups (Table S3). Two-way ANOVA on ICSS thresholds during precipitated withdrawal revealed a significant main effect of group ($F (3, 22) = 6.2$, $p = 0.003$), but no significant effect of session or interaction. Holm-Sidak's multiple comparison showed significantly higher ICSS thresholds in the MOR + NX group compared to the SAL + SAL control group during all sessions (all $t(22) \geq 3.71$, all $p < 0.05$) (Figure S3A). There was no significant effect of group, session, or group x session interaction on ICSS latencies during precipitated withdrawal (data not shown).

Spontaneous withdrawal: ICSS

Baseline ICSS thresholds and response latencies did not differ between groups (Table S3). ICSS thresholds during hours 2 (agonist effect) and hours 6 – 98 (withdrawal period) of spontaneous withdrawal did not differ between the two groups receiving MOR (MOR + NX versus MOR + SAL groups) or SAL (SAL + SAL versus SAL + NX groups). Data from these groups were therefore combined into a single MOR (n = 15) and SAL (n = 5) group. Welch's corrected t test showed no significant difference in ICSS thresholds between MOR (118.4 ± 14.03%) and SAL (102.6 ± 2.58%) groups during the 2-hour session, indicating an absence of effects of MOR itself on ICSS. Two-way ANOVA on ICSS thresholds during spontaneous withdrawal 6-98 hours after morphine injection revealed no significant main effect of time, group (morphine vs. saline) or interaction (Figure S3B). However, comparison of peak ICSS threshold values between 6 hours and 98 hours (regardless of the time point at which they occurred) differed significantly between the morphine (121.7 ± 4.78%) and saline (106.8 ± 2.04%) groups (Welch-corrected $t(17.54) = 2.85$, $p = 0.01$). No significant difference in ICSS response latencies was observed between groups 2 hours after injection (agonist effect) or 6-98 hours after injection (withdrawal effect) (data not shown).

Precipitated withdrawal: Somatic signs

One-way ANOVA revealed a significant overall difference in somatic signs scores between groups during the 5th session of precipitated withdrawal during late stage dependence ($F(3, 23) = 4.57$, $p = 0.01$). However, Dunnett's multiple comparison indicated that none of the groups differed significantly from the SAL + SAL group (Figure S3C).

Spontaneous withdrawal: Somatic signs

Somatic signs did not differ between the two groups receiving MOR or between the two groups receiving SAL. Data from these groups were therefore combined into single MOR (n = 15) and SAL (n = 5) conditions, respectively. Total somatic signs in the MOR condition were significantly higher than in the SAL condition (Welch-corrected $t(21.44) = 2.19$, $p = 0.04$) (Figure S3D).

Correlations in the MOR + NX group during late-stage dependence

ICSS thresholds or somatic signs scores during late-stage dependence during precipitated or spontaneous withdrawal did not correlate with any MSA measure, except for a significant correlation between higher composite z-score for ICSS thresholds during precipitated withdrawal and higher infusions during extinction (i.e., *greater* resistance to extinction; $r = 0.61$, $p = 0.04$).

Comparison of withdrawal severity during acute dependence and late-stage dependence in rats that completed both phases suggested that these measures were largely independent. Thus, ICSS thresholds during precipitated or spontaneous withdrawal during late-stage dependence did not correlate with these same ICSS measures during acute dependence (all r: $-0.16 \leq r \leq -0.04$, all $p \geq 0.57$). Somatic signs during spontaneous withdrawal correlated significantly between late-stage dependence and acute dependence ($r = 0.73$, $p = 0.007$), but this correlation was not observed during precipitated withdrawal ($r = 0.13$, $p = 0.45$).

Table 4

Variables	1. Precip WIA	2. Spont. WIA	3. Acquisition	4. Log α	5. Extinction	6. MOR+ CUE	7. MOR+ NO CUE	8. YOH+ CUE	9. YOH + NO CUE
1. Precip. WIA	–								
2. Spont. WIA	.41*	–							
3. Acquisition	-.39*	-.55**	–						
4. Log α	.43*	.40	-.42*	–					
5. Extinction	-.01	.-.26	.35	-.39	–				
6. MOR+CUE	-.07	-.53**	.15	-.13	.20	–			
7. MOR + NO CUE	-.21	-.63**	.33	-.21	.35	.81***	–		
8. YOH + CUE	-.32	-.17	.18	-.57**	.35	-.13	-.05	–	
9. YOH + NO CUE	.11	.04	-.12	-.26	-.20	-.16	-.26	.61**	–
10. VEH+ CUE	-.17	-.06	.16	-.66***	.38*	-.10	-.11	.77***	.59**

Correlation between ICSS withdrawal measures and MSA measures (Pearson's R) in the Mor + NX group. 1. Standardized composite score of average WIA, peak WIA, and degree of sensitization of WIA during naloxone-precipitated withdrawal. 2. Peak ICSS threshold during spontaneous withdrawal. 3. Average infusions during first 10 days of acquisition. 4. Log-transformed elasticity of demand computed from exponential demand model. Higher log α = lower reinforcement efficacy. 5. Average infusions during first 10 days of extinction; 6.-10. Reinstatement score (active – inactive lever pressing) during reinstatement induced by morphine with cue (MOR+CUE), morphine with no cue (MOR+ NO CUE), yohimbine with cue (YOH+CUE), yohimbine with no cue (YOH+ NO CUE) and cue alone (averaged across VEH + CUE condition during morphine- and yohimbine-induced reinstatement testing).*,**,*** $p \leq 0.05$, 0.01, 0.001.

Discussion

Greater WIA during antagonist-precipitated and spontaneous withdrawal in an acute dependence model was associated with lower vulnerability on multiple measures of subsequent i.v. MSA (e.g., elasticity of demand, reinstatement). In fact, WIA predicted a wider range of MSA measures than did any individual measure of MSA. These findings are consistent with the principle that initial drug sensitivity is an important predictor of subsequent drug use (Deminiere et al., 1989; Chappell & Weiner, 2008; Nishida et al., 2016), and also support the notion that drug withdrawal sensitivity may be protective against drug addiction (Carroll et al., 2008; Holtz et al., 2015; Radke et al., 2015). In particular, our findings with outbred rats complement findings of lower WIA in rats bred for high saccharin consumption (Holtz et al., 2015), a line that exhibits greater SA of opioids and other drugs (Carroll et al., 2002). Together, these data identify WIA as a potential target for understanding behavioral and neurobiological mechanisms underlying the emergence of opioid addiction.

Several features of WIA during acute dependence distinguish it from other behavioral measures of vulnerability to addiction-like behaviors. First WIA is unique in that it reliably predicts individual differences in opioid SA in outbred rats, whereas numerous established behavioral markers of individual differences in stimulant and alcohol SA (e.g., sensation-seeking as measured by open-field locomotor activity, impulsivity) do not (Study 1; Dileen et al., 2012; McNamara et al., 2010). Indeed, open-field activity was not correlated with any measure of MSA in this study (see Supplementary Material), consistent with our previous findings using a more limited set of MSA measures (Study 1).

WIA also differs from other behavioral predictors of SA of other drugs in that it predicted a variety of measures of SA (Belin et al., 2016). An additional unique feature of WIA is that it is an outcome of early opioid exposure, as opposed to a preexisting disposition. Therefore, WIA may represent a neuroadaptive *mechanism* underlying addiction vulnerability, as opposed to only a behavioral *indicator*. As such, WIA promises to provide unique information on addiction vulnerability to complement findings obtained using existing behavioral markers of addiction vulnerability.

In contrast to WIA, somatic signs during acute dependence did not predict any primary MSA measure. These data complement previous findings indicating that affective/emotional and somatic withdrawal signs are mediated by distinct neurobiological mechanisms (Koob & Le Moal, 1997; Nestler & Carlezon, 2006), and supports the notion that the former have greater relevance to addiction vulnerability (Schulteis et al., 1994; Baker et al., 2004; Koob & Le Moal, 2005).

The current findings contrast with some studies reporting a *positive* relationship between withdrawal sensitivity and addiction vulnerability (Ahmed et al., 2002; Kenny et al., 2006; Funk et al., 2006). Numerous methodological differences between studies could account for this discrepancy (e.g., drug class studied, etc). In addition, the current acute dependence model isolates the earliest stages of dependence, while prior studies involved subjects in which dependence had already been established. As such, withdrawal sensitivity may shift from being a protective factor to a vulnerability factor for addiction as dependence develops (Kiluk et al., 2019). The fact that WIA during late stage dependence was associated with *greater* resistance to extinction (see Supplemental Materials) may be

consistent with this possibility. The lack of correlation between WIA during acute dependence and late-stage dependence (Supplementary Material) also suggests that each of these stages may provide unique information. Use of larger group sizes in order to provide adequate statistical power is needed to further address this issue, which was not a primary goal of this study.

Comparison of locomotor activity and MSA in the MOR + NX and control groups suggests that a history of morphine exposure and/or withdrawal had limited or no effects on these measures. The MSA data contrast with findings that repeated, experimenter-administered acute drug injections (and presumably spontaneous withdrawal episodes) can enhance subsequent drug SA (Piazza et al., 1989; Mendrek et al., 1998; Shoaib et al., 1997). Methodological differences across studies (e.g., types of drugs, duration of interval between the final acute injection and onset of SA) may account for the similar MSA across groups in this study. Importantly, results from this secondary comparison do not impact interpretation of the correlations between WIA and measures of MSA in the Mor + NX group, which was our primary outcome.

Behavioral economics has been useful for understanding individual differences in addiction vulnerability in both humans and animals (Chase et al., 2013; Worley et al., 2015; Diergaarde et al., 2008; Grebenstein et al., 2013; Hursh & Silberberg, 2008; LeSage et al., 2016), but has not been applied extensively to MSA. Consistent with Study 1, an exponential demand function generally provided a good fit for morphine consumption under an FR escalation procedure. There were also considerable individual differences in α (reinforcing efficacy) that were correlated with severity of WIA during precipitated

withdrawal, and a similar trend was observed for spontaneous withdrawal. Together, these data further support the utility and sensitivity of behavioral economics to study individual differences in opioid addiction vulnerability.

A potential limitation of this study is that all rats underwent the experimental phases in a fixed order. It could therefore be argued that the relationships we observed were due to the order in which the measures were assessed, rather than their relationship *per se*. It is important to note that not all aspects of the protocol were fixed, as order of reinstatement conditions (drug + cue, cue alone, etc) within each reinstatement phase (morphine/cue, yohimbine/cue) were counterbalanced across rats. In addition, the current order of phases was to some extent unavoidable due to the goals of the study (e.g., assessment of acute dependence prior to MSA) or to the required procedure for assessing certain MSA measures (e.g., testing extinction of MSA prior to reinstatement). Also, it was prudent to test yohimbine-induced reinstatement as the final MSA measure because of the well-established long-term effects of yohimbine or other stressors on behavior, including drug sensitivity (Ball et al., 2015; Barsy et al., 2011; Pizzimenti et al., 2017). Nonetheless, we cannot rule out the possibility that the relationships observed here were specific to this order of experimental phases. However, even such an order effect would not negate the utility of the current procedure.

In conclusion, this study establishes WIA as one of the first behavioral measures to reliably predict individual differences in future opioid SA. Evaluating the generality of the current findings to other subject variables (e.g., genetic background using inbred rat strains), opioids (e.g., heroin, fentanyl), withdrawal measures (e.g., other measures of

anhedonia such as sucrose preference, or other negative affective withdrawal signs such as elevated startle responding) will be an important direction for future research. Given the numerous sex differences in addiction vulnerability reported in both humans and animals (Back et al., 2011; Becker & Koob, 2016; Becker et al., 2017), evaluating effects of sex in this model is also of interest. Furthermore, measures of opioid SA that model other aspects of addiction (e.g., escalation of SA under extended access conditions, a model of compulsive drug use,) are also an important direction for future research. Finally, characterizing the neurobiological mechanisms underlying WIA will allow addiction-related effects (i.e., those uniquely related to severity of WIA) to be differentiated from other, corollary effects of opioids. Hence, further use of this model promises to provide novel insights into neurobiological mechanisms underlying vulnerability and/or resilience to opioid addiction.

Chapter 4: Individual Differences in Different Measures of Opioid Self-administration in Rats Are Accounted for by A Single Latent Variable

Introduction

Individual differences in susceptibility to addiction in humans have been studied widely through factor analysis, a statistical method that identifies "latent" variables (variables that are not measured directly) that reflect the common variance among a larger number of related measures. These models provide both insights into the relationship between different facets of addiction- and dependence-related symptomatology (Gillespie et al. 2007), and a relatively parsimonious account of disease comorbidity (Neale & Kendler, 1995). For example, factor analytic approaches have revealed that liability to alcohol abuse is associated both with a general drug abuse vulnerability factor and with several factors that are specific to this form of addiction (e.g., genetic variants in alcohol metabolizing enzymes) (Krueger et al., 2002; Tsuang et al., 1998; Luczak et al., 2006).

Factor analytic approaches have been widely used in the clinical literature to explore the factor structure underlying various addiction measures. Such structures may have both vertical and horizontal dimensions. The vertical dimension essentially represents hierarchical relationships between concrete traits or measures and higher-order, more abstract, or general "latent" factors. The horizontal dimension represents the degree of similarity between factors within a single level of the hierarchy (Goldberg & Velicer, 2006). Elaboration of such two-dimensional factor structure may yield one or more robust endophenotypes that can be used to identify genomic loci associated with core features of substance use disorders (Hicks et al., 2010; Palmer et al., 2014).

In animal addiction research, factor analytic approaches could be useful in identifying the underlying associations between, and uniqueness of, different addiction-related behavioral measures, developing more reliable measures of addiction, and uncovering their underlying genomic and neurobiological substrates (see Chapter 1). Factor analytic approaches have rarely been employed in this area, however, despite the fact that the drug self-administration (SA) paradigm models a variety of aspects of addiction (e.g., acquisition, relapse, etc.) within individual subjects, thereby lending itself to multivariate statistical analyses. In one previous study, an exploratory factor analysis revealed three addiction vulnerability measures – (a) SA despite punishment, (b) progressive ratio (PR) breakpoint, and (c) drug-seeking during no-drug periods – as loading onto a single latent factor underlying cocaine SA in rats, whereas extinction loaded onto a separate factor (Deroche-Gamonet et al., 2004). Nevertheless, there has yet been any preclinical SA study to test how well drug SA data fit any hypothesized factor structure. Moreover, despite the dramatic impact of opioid addiction on public health (Center for Behavioral Health Statistics and Quality, 2020), no preclinical studies have applied FA to opioid SA.

The primary goal of this study was to use FA to examine the latent factor structure between four measures of i.v. opioid SA in rats (e.g., acquisition, demand elasticity, morphine/cue-induced reinstatement, stress/cue-induced reinstatement), using data from Study 2. The four SA measures were selected due to their common use in preclinical studies and to the relevance of each to different aspects of addiction (Belin et al., 2008; Banna et al., 2010; Sinha, 2001).

In animal research, there is frequently the implicit assumption that a variety of SA variables all have relevance to addiction vulnerability. This is consistent with findings in humans showing that individual differences in multiple measures of abuse liability are best accounted for by a single latent factor (Blanco et al., 2013; Lennox et al., 2006; Lynskey & Agrawal, 2007). Therefore, in the current study, a one-factor model was fitted to the data, with the single latent factor conceptualized as the "addiction" factor.

It has been proposed that minimum sample size required for a factor analysis ranges between N = 50-250 (de Winter & Dodou, 2012; Cattell, 1978; Gorsuch, 1983; Guilford, 1954; Kline, 1979). Conducting small sample size FA might result in many issues that are otherwise uncommon in large sample size analyses, such as Heywood cases denoting negative estimated variances (de Winter & Dodou, 2012; Cooperman & Waller, 2021). Preclinical addiction studies have typically employed relatively small sample sizes, which pose a challenge to the use of FA. The secondary goal of the current study was to test the utility of a novel approach to conducting FA on preclinical data that allows for smaller sample sizes to be used. Several proposed (Jacobucci et al., 2016; Jung & Takane, 2007; Jung & Lee, 2011) "regularization methods" can effectively address the challenges of conducting small sample FA by reducing the number of estimated model parameters. In this study, we utilized two robust regularization methods in conjunction with a method to obtain a robust correlation matrix from our data (Rousseeuw & Driessen, 1999) to demonstrate the feasibility of conducting FA in a small preclinical dataset. By applying these iterative statistical procedures to our data, we aimed to understand the core dimensions underlying the morphine SA model.

Materials and Methods

Overview of experimental protocol

Data from Study 2 were used for the current analyses. Male Sprague-Dawley rats were first trained in an intracranial self-stimulation (ICSS) paradigm and then underwent naloxone-precipitated and/or spontaneous withdrawal from acute morphine injections or received control (saline) injections. During the subsequent MSA protocol, all rats first acquired MSA, then underwent demand testing in which the fixed ratio (FR) response requirement was progressively increased. Rats then re-acquired and subsequently underwent extinction of MSA. After extinction, rats were tested for drug-induced reinstatement (with morphine injection prior to the SA session) and finally, stress-induced reinstatement (with injection of the pharmacological stressor yohimbine prior to the SA session), both in the presence and absence of the visual cue paired with morphine, and with appropriate within-subject control conditions (see Chapter 2 for more details on animals, apparatus and experimental protocol). Since a history of morphine and/or naloxone injections during ICSS testing did not have a significant effect on subsequent MSA, rats from all groups that completed all phases of the study were included in the data analyses (N = 43).

Overview of factor model

We tested a one-factor model with one latent variable (the "addiction" factor) and four observed variables from the MSA model: acquisition, elasticity of demand (α), and morphine/cue- and stress/cue-induced reinstatement with visual cue light present. These measures were chosen due to the distinct aspects of addiction-like behavior they are often

thought to capture and their common application in drug SA research. Acquisition was defined as the average number of infusions across the first 10 days of MSA. An exponential demand function was fitted to data from the FR escalation protocol to obtain the α statistic, as described in Studies 1 & 2. α refers to the rate of change in consumption with increases in unit price (elasticity of demand), with higher α values indicating lower reinforcement efficacy. Reinstatement was measured as the difference between the number of active and inactive lever presses over each of the 2-hr reinstatement test sessions after the challenge (i.e., morphine or yohimbine) drug injection, with cue light present. These reinstatement conditions were analyzed because they produced more robust reinstatement than either the challenge drug (morphine or yohimbine) alone or the cue alone (see Fig 12C and 12D). A higher number of infusions during acquisition, lower elasticity of demand (α), and higher reinstatement scores reflect greater abuse liability for each of these measures.

Statistical analyses

All statistical analyses were performed in GraphPad Prism (GraphPad Software, San Diego, California USA) and R ver. 4.0.4 (R Core Team, 2021). A one-factor model was hypothesized to show good model-fit with each of the SA measures showing high factor loadings, indicating a common "addiction" factor underlying all tested SA measures.

Three distinct methods were used for extracting factor loadings. Given the small sample size of our data set and the several outlying values (to be discussed later), we used two distinct factor extraction algorithms that are known to yield robust factor loadings in small sets of non-normal data. The first method involves computing Mahalanobis distances for all data points and then identifying the number of multivariate outliers via a series of

chi-squared tests ($\alpha = 0.1$). Next, we used the minimum covariance determinant (MCD: MASS package; Venables & Ripley, 2002; Rousseeuw & Driessen, 1999) method to produce a robust estimator of multivariate scatter and center to remove the multivariate outliers and generate a robust correlation matrix. This robust correlation matrix was factor analyzed with a regularized least squares estimator (fareg function; Waller, 2020). Robust least squares estimation does not assume data multinormality and aims to minimize residuals between the observed and reproduced correlations under the proposed factor model (Beauducel & Herzberg, 2006). Model fit was tested via the correlation root mean square residual (CRMR) statistic:

$$CRMR = \sqrt{\frac{1}{t-p}\sum_{i<j}(\rho_{ij} - \rho_{ij}^{0})^2},$$

with t denoting the number of nonredundant population variances and covariances among the p observed variables, ρ_{ij} denoting the correlation between variables i and j, and ρ_{ij}^{0} denoting the model implied population correlation under the theoretical model (Maydeu-Olivares, Shi & Rosseel, 2018). CRMR is commonly used in FA and structural equation modeling (SEM) as a model fit statistic, with smaller numbers indicating better model fit. Finally, effect size of overall model misfit was determined by the Γ_1 statistic, defined as

$$\Gamma_1 = \frac{p}{tr(\Sigma\Sigma_0^{-1})^2},$$

where Σ denotes a population covariance matrix, Σ_0 denotes the population covariance under the null hypothesis, and *tr* denotes the trace operator (the trace of a square matrix equals the sum of its diagonal elements).

The second robust method for analyzing the data used regularized factor analysis as described by Jung and colleagues (Jung & Takane, 2007; Jung & Lee, 2011; these methods are implemented in the fareg function; Waller, 2020). Both least squares (LS) and maximum likelihood (MLE) regularized FA were used to estimate robust factor loadings for testing the 1-factor model. Previous work suggests that these methods work extremely well in small samples of nonnormally distributed data (Copperman & Waller, 2021; Jung & Takane, 2007; Jung & Lee, 2011) and thus were deemed ideal for the current study.

To further demonstrate the advantages of the robust correlation and robust factor analytic methods, a third analysis was implemented using principal axis factoring, a traditional factor extraction method, with the complete data set of 43 rats (using the faMain function in the R fungible library; Waller, 2020). This method was not expected to perform well given the small sample size of our data set and the existence of several multivariate outliers. To allow comparison with the other analyses, we also computed the CRMR index for this analysis.

Results

MSA

Detailed behavioral results from the MSA protocol are reported in Study 2. Briefly, rats reliably acquired MSA, exhibiting a clear preference for the active over inactive response lever (Fig 12A). Increases in FR requirement resulted in a progressive reduction in morphine consumption that was well-described by an exponential demand function (R^2 = 0.84) (12B). After MSA reacquisition and extinction in the absence of the morphine-associated cue light, rats reliably reinstated active lever responding following a priming

dose of morphine in the absence of the cue light (MOR + NO CUE; 12C), response-contingent presentation of the cue light (VEH + CUE), or combined exposure to morphine and the cue light (MOR + CUE). Similar findings were observed when reinstatement was induced by the pharmacological stressor yohimbine (12D).

FA

Each variable was standardized to keep their scales consistent. The factor loadings from each analysis are shown in Table 4.

For the two regularized FA analyses, 5 multivariate outliers ($\alpha = 0.1$) were identified from the chi-squared test using Mahalanobis distance. Subsequently, these 5 multivariate outliers were excluded from the robust correlation matrix computation using MCD (Rousseeuw & Driessen, 1999). Using the robust correlation matrix with LS estimation, the first regularized FA revealed that acquisition, elasticity of demand and morphine/cue-induced reinstatement showed high factor loadings (all $|\text{loadings}| \geq 0.58$) on a single common factor whereas stress/cue-induced reinstatement showed low factor loading on this dimension (loading = 0.27) (Table 5). The second regularized FA using MLE factoring (on the same robust correlation matrix) produced similar results. Acquisition, elasticity of demand and morphine/cue-induced reinstatement showed high factor loadings on a single dimension (all $|\text{loadings}| \geq 0.59$), and stress/cue-induced reinstatement again showed a low factor loading (loading = 0.28) (Table 5). Overall, based on the CRMR and Γ_1 values, the one-factor model showed excellent model fit (CRMR = 0.03, Γ_1 = 1 for both analyses).

Figure 12. Active and inactive lever pressing during acquisition (n = 43) (A); exponential demand curve for morphine intake during demand testing (n = 42) (B); difference scores between active and inactive lever pressing during morphine-induced (n = 43) (C) and yohimbine-induced (n = 43) reinstatement (D). MOR = morphine. YOH = Yohimbine. VEH = Vehicle. Data points represent mean ± SEM. *: significant difference compared to VEH+NO CUE responding, $p < 0.05$, ***: $p < 0.001$.

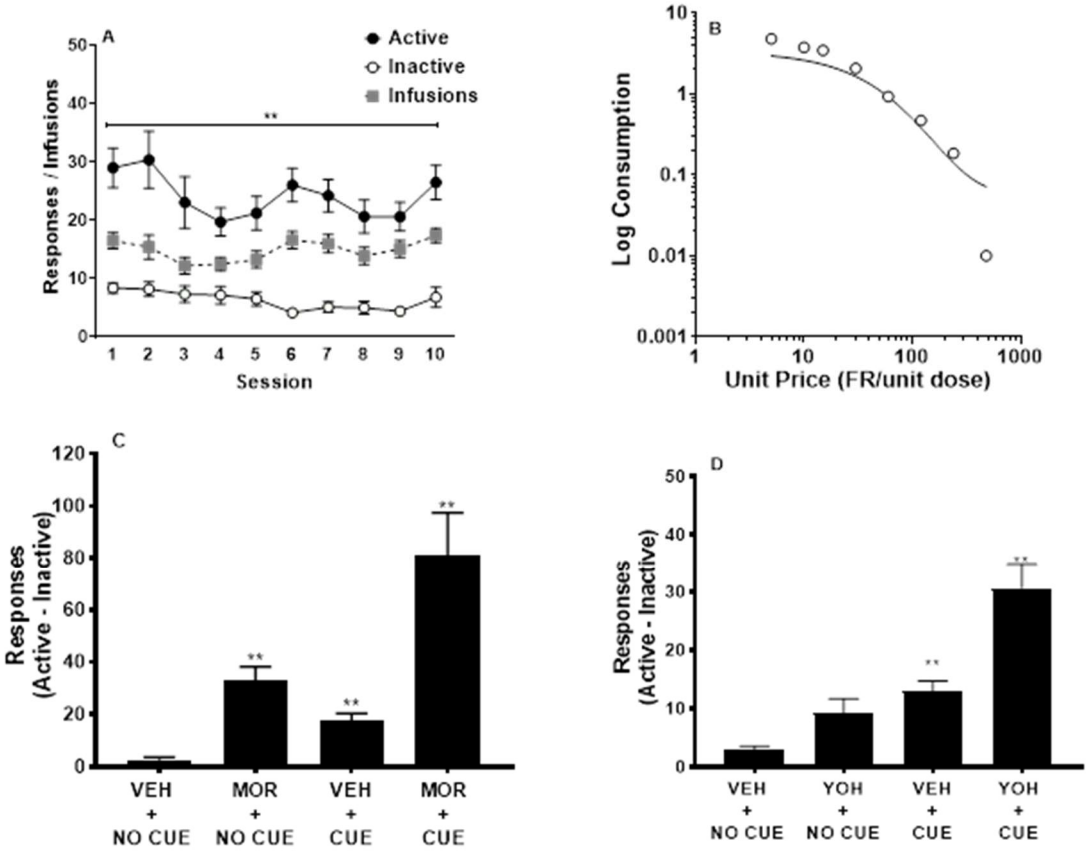


Table 5

	Factor Loadings		
SA Measures	Robust LS	Robust MLE	Principal Axis
Acquisition	0.58	0.59	0.48
Demand	-0.63	-0.64	-1.03
Morphine/cue-induced reinstatement	0.62	0.63	0.32
Stress/cue-induced reinstatement	0.27	0.28	0.27

Estimates for factor loadings from 3 independent analyses. Robust LS: regularized FA using least squares estimates with MCD robust correlation matrix excluding 5 multivariate outliers; robust MLE: regularized FA using maximum likelihood estimates with robust correlation matrix excluding 5 multivariate outliers; principal Axis: traditional principal axis factor extraction.

Discussion

Our data demonstrated that a single latent Addiction factor fits four distinct morphine SA measures. This indicates that acquisition, elasticity of demand, morphine/cue-induced reinstatement, and stress/cue-induced reinstatement all in some way measure a common construct, akin to a general factor of addiction vulnerability. These findings support the implicit assumption in the preclinical literature that these different SA measures are related to abuse liability. This one-factor model is consistent with the clinical literature that also posits a single latent factor to underlie multiple measures of addiction (Blanco et al., 2013; Lennox et al., 2006; Lynskey & Agrawal, 2007).

In terms of individual factor loadings, results from both regularized FAs implicated elasticity of demand as the variable most reliably strongly associated with the Addiction factor, with a stable, high factor loading across both analyses. Previous studies have demonstrated the value of behavioral economics in studying individual differences in opioid addiction vulnerability in both humans and animals (Worley et al., 2015; Study 1 & 2), and the high factor loading from our results further demonstrates the utility of this demand function.

In contrast, stress/cue-induced reinstatement did not load onto the Addiction factor. This means that although this factor underlies the four SA measures overall, other, unique factors likely contribute more strongly to individual differences in levels of stress/cue-induced reinstatement than to the other three SA measures. The unique factor in this case may be the influence of stress itself. While activation of stress systems contributes to various facets of addictive behavior measured in the SA paradigm (e.g., acquisition –

Piazza & Le Moal, 1998; Shaham et al., 1992), individual differences in sensitivity to stress (e.g., conferred by polymorphisms of the corticotropin releasing factor (CRF)1 receptor or CRF binding protein (Hansson et al., 2016; Treutlein et al., 2006) may well have their largest impact on stress/cue-induced reinstatement (George & Koob, 2010).

Utilizing different regularized FA methods with robust correlations in direct comparison with a traditional principal axis factoring method, we have demonstrated the feasibility of these statistical tools in analyzing sample sizes that are realistic targets for preclinical studies where traditional factor analysis methods might fail. These methods help address some major statistical challenges in small sample size factor analyses such as Heywood cases, which was observed using the traditional factor extraction method (Heywood, 1931; Kolenikov & Bollen, 2012). Moreover, the regularization methods used in the current study have been shown to provide good recovery of underlying factor structures in simulation data, increasing confidence in the interpretation of our results (Jung & Takane, 2007; Jung & Lee, 2011).

Though statistical methods such as regularization enable complex multivariate analyses of small sample sizes, there are inherent limitations of such analyses, such as sampling bias, that could not be fully addressed in this study. Future studies could include a larger preclinical sample for analyses where cross-validation is warranted, such as regularized factor analytic methods using least absolute shrinkage and selection operator (LASSO) penalization (Jacobucci et al., 2016; Tibshirani, 1996). Additionally, with a larger preclinical sample, a higher count of observed variables could be included in the model, allowing for examination of more complex multi-factor models.

A further limitation of this study is that some rats had prior morphine and/or naloxone experience, and all rats underwent ICSS surgery and training. However, no significant difference was found on any SA measure between rats with morphine and/or naloxone experience compared to saline controls. Furthermore, despite their history of ICSS testing, rats from the current study showed similar acquisition and demand compared to rats from a previous study that did not have a history of ICSS testing (Studies 1& 2). Notwithstanding these limitations, the current study represents a first step in using FA to understand the factor structure of opioid SA.

Overall, this study identifies a single factor that contributes to four common opioid SA measures, revealing the common and unique information each of the measures could contribute to preclinical addiction literature. Elasticity of demand most reliably represents the common "addiction" factor while stress/cue-induced reinstatement provides information about constructs other than the common factor tested in this model. Therefore, future studies examining individual differences in opioid SA may be rendered most informative by selectively examining demand and stress/cue-induced reinstatement. More generally, exploring relationships beyond prevailing bivariate correlations in preclinical behavioral studies may further our understanding of addiction vulnerability and its neurobiological basis and lead to better prevention and treatment.

Chapter 5: Summary and Overall Conclusions

Among all individuals who experiment with opioids, only a small subset develops opioid addiction. Furthermore, once acquired, the severity of addiction varies considerably between individuals (American Psychiatric Association, 2013; Belin et al., 2016; Vowles et al., 2015). Insight into the behavioral predictors of individual differences in the vulnerability to opioid addiction and the severity of addiction once acquired could help uncover psychological, neurobiological, and genetic factors contributing to opioid addiction, and could be key to developing effective preventions and treatments.

How Do We Identify Behavioral Predictors of Individual Differences in Opioid Addiction Vulnerability?

Despite the importance of identifying behavioral predictors of individual differences in opioid addiction vulnerability, very few behavioral predictors of opioid SA have been established in preclinical research. In this dissertation, we utilized several unique approaches to evaluate several potential behavioral predictors of opioid SA in rats.

Approach #1: Evaluate Behavioral Predictors of Individual Differences in Addiction to Other Drugs of Abuse

A logical starting point in identifying predictors of opioid SA is to evaluate predictors of SA of other drugs of abuse (e.g. cocaine) (Belin et al., 2011; Belin et al., 2016; Suto et al., 2001; Turner et al., 2008; Piazza et al., 2000). To this end, Study 1 evaluated the ability of spontaneous locomotor activity, which predicts SA of stimulants such as cocaine, to predict opioid SA. Contrary to our hypothesis, spontaneous locomotor activity did not predict subsequent acquisition or demand of morphine SA. These results

complement previous preclinical studies showing that impulsivity, another reliable behavioral predictor of SA of other drugs, did not predict opioid SA (McNamara et al., 2010). Together, these findings support the notion that distinct factors may contribute to individual differences in opioid addiction compared to other drugs of abuse. Previous research has shown many differences between the neurobiological mechanisms between drugs such as psychostimulants and opioids. For example, most neurons in the medial prefrontal cortex and the nucleus accumbens encode rewarding effects of either heroin cocaine, instead of both (Badiani et al., 2011; Ettenberg et al., 1982; Pettit et al.,1984; Chang et al., 1998). Such differences could potentially account for the unique predictors of SA of opioids versus psychostimulants.

Approach #2: Evaluate Novel Predictors of Individual Differences in Opioid Addiction Vulnerability, Including Those Related to Opioid Exposure Itself

The findings of Study 1 suggested the need for other approaches for identifying behavioral predictors of opioid SA. Sensitivity to the acute drug effects can be an important predictor of drug addiction in human literature, and sensitivity to the analgesic effects of opioids is one of the few behavioral measures shown to predict opioid SA in rats (DiFranza et al., 2007; O'Loughlin et al., 2003; Schuckit et al., 2004; Nishida et al., 2016). Therefore, we evaluated whether another consequence of acute opioid exposure – anhedonia during withdrawal – could also predict opioid SA. Results of this study revealed a negative relationship between withdrawal-induced anhedonia (WIA) during initial morphine exposure and multiple measures of subsequent morphine SA. These results established

WIA as one of the very few behavioral predictors identified in the context of opioid SA (Nishida et al., 2016; McNamara et al., 2010; Study 2).

Given that opioid withdrawal includes a variety of symptoms, it is possible that not all withdrawal sensitivity symptoms could be protective against opioid addiction. In Study 2, somatic signs of withdrawal did not predict subsequent morphine SA, which suggests that affective withdrawal could be more relevant to opioid addiction vulnerability. However, other affective withdrawal symptoms, such as anxiety, might play a different role in opioid addiction vulnerability compared to initial WIA. This idea is supported by previous research showing that saccharin-preferring (higher propensity to drug SA) rats exhibited lower anhedonia but higher anxiety during drug withdrawal (Holtz et al., 2015; Radke et al., 2013).

Approach 3: Evaluate Vulnerability to Distinct Stages of Opioid Addiction

Since different measures of opioid SA each model a unique aspect of opioid addiction, it could be worthwhile to further investigate how each stage of opioid SA differs, as well as the factors contributing to individual differences to each stages of opioid SA. Study 2 provided some initial insight into the distinct stages of morphine SA by showing that WIA during naloxone-precipitated withdrawal and spontaneous withdrawal each predicted some but not all stages of morphine SA. Additionally, Study 3 revealed that despite being described by a common latent Addiction factor, acquisition, elasticity of demand, morphine-induced reinstatement and stress-induced reinstatement all had a large proportion of their variability not accounted for by the Addiction factor. The idea of distinct stages of SA is also supported by other studies examining multiple drug SA measures, and

found that measures such as cue-induced reinstatement and extinction did not relate to the same underlying factors as other SA measures in those studies did (Flagel et al., 2016; Deroche-Gamonet et al., 2004). Further research into these unique stages of opioid SA could provide valuable information for prevention and treatment development for targeted behaviors (e.g., early-stage addiction or stress-induced relapse behavior).

Approach 4: Establish Reliable and Valid Measures to Study Opioid Addiction Vulnerability

In order to understand both the common and unique predictors of individual aspects of opioid addiction behavior, it is vital to establish a solid foundation for modeling these behaviors in preclinical research. The flexibility of the SA paradigm allows a diverse array of addiction-related behavior to be measured with a wide range of behavioral measures, but at the same time makes it difficult to determine which behaviors to model, or how to measure those behaviors. Multivariate statistical methods, including the FA utilized in Study 3, can help address this issue by providing insight on both how various measures relate to each other, as well as how they represent underlying constructs such as addiction. Moreover, to increase replicability of preclinical findings and choose the ideal SA measures for each study, it could be beneficial to examine the reliability of each SA measure (e.g., how a measure performs across different experiments).

One reliable SA measure identified in the current studies in elasticity of demand as determined using behavioral economics. The exponential demand function described morphine consumption well regardless of whether unit price was manipulated via reductions in morphine unit dose or increases in response requirement. Moreover, various

measures (α, Q0, etc.) from the behavioral economics model all showed significant between-subject variability in Study 1 and Study 2, making them suitable for individual differences research. Finally, among the measures included in the one-factor model in the FA, elasticity of demand reliably loaded closely to the Addiction factor in both regularized analyses, making it a good indicator of the latent Addiction factor. Additionally, other studies have shown the feasibility of using behavioral economics to measure reinforcing efficacy of other opioids in animals as well as reinforcing efficacy of opioids in humans (Worley et al., 2015; Stafford et al., 2019; Strickland et al., 2019). As such, behavioral economics could therefore not only be a reliable measure to use with the SA paradigm in rats, but also a bridge to narrow the gap between preclinical and clinical studies.

Approach #5: Evaluate the Feasibility of Using Novel Statistical Techniques to Study Opioid Addiction Vulnerability in Small Samples

FA has been used frequently in clinical addiction research involving large sample sizes. However, sample sizes comparable to those typically used in clinical research are often difficult to accomplish in preclinical studies. The small sample size in typical preclinical behavioral studies poses many challenges that novel statistical methods aim to address, as demonstrated by the comparison between a traditional factor extraction method and robust FAs in Study 3. The traditional principal axis factor extraction method produced a Heywood case, an error many small sample size analyses run into where an impossible solution such as negative variance was found. In contrast, the two regularized FA methods with robust correlation matrices successfully estimated factor loadings for each SA measure. These findings showed that with novel statistical methods addressing some

limitations with small sample size analyses, preclinical studies on individual differences in opioid addiction vulnerability could also start exploring complex multivariate relationships within the SA paradigm, potential latent factors underlying opioid addiction vulnerability, and eventually behavioral predictors of these latent factors.

Additional Future Directions

Findings from studies described in this dissertation raise many additional research questions to be addressed in future studies. The role of WIA during initial opioid exposure has been established in this dissertation in male rats only. Nevertheless, women can develop addiction to opioids and other drugs more rapidly and relapse more easily than men, and females can be more vulnerable to addiction-related effects of drugs of addiction compared to males in preclinical models (Lynch et al., 2002; Hernandez-Avila et al., 2004; Husky et al., 2008). Therefore, examining the ability of WIA to predict opioid SA in females could help uncover the common and unique mechanisms underlying opioid addiction vulnerability in males and females.

To further understand the differences between the role of sensation-seeking in addiction to opioids versus other drugs, future studies could focus on more direct comparisons using SA of both opioids and other drugs within the same animals, and to examine individual differences in neurobiological pathways that are uniquely impacted by each drug (Badiani et al., 2011). Moreover, it could be beneficial to explore the similarities and differences in mechanisms underlying stress-induced reinstatement and other, similar SA measures, such as morphine-induced reinstatement. Understanding the mechanisms for those similarities and differences could provide valuable insight into this important relapse

behavior and subsequently shed light on effective preventions for relapse. Finally, continue employing multivariate statistics and refining analytical methods for small sample size analysis could shed light on more complex relationships between behavioral predictors, SA measures and underlying latent factors related to opioid addiction, and eventually lead to better understanding of mechanisms contributing to opioid addiction vulnerability.

Final Conclusions

Overall, these studies extend the opioid individual differences literature by 1) showing that locomotor activity, a reliable predictor of SA of other drugs of abuse, does not predict opioid SA, 2) identifying WIA during initial drug exposure as a reliable predictor of opioid SA, and 3) applying FA and statistical methods appropriate for small sample size preclinical studies to opioid SA data. Future preclinical studies could benefit not only from examining behavioral predictors more relevant to opioid addiction vulnerability such as initial WIA, but also from identifying reliable and valid measures of opioid addiction behaviors such as elasticity of demand under the SA paradigm. Understanding factors contributing to individual differences in opioid addiction vulnerability could eventually lead to the development of better preventions and treatments for opioid addiction.

Bibliography

Ahmed SH, Kenny PJ, Koob GF, Markou A (2002) Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. Nat Neurosci 5: 625-6.

Ahn, W.Y., Vassileva, J., 2016. Machine-learning identifies substance-specific behavioral markers for opiate and stimulant dependence. Drug Alcohol Depend 161, 247-257.

Allahverdiyev O, Turkmen AZ, Nurten A, Sehirli I, Enginar N (2015) Spontaneous withdrawal in intermittent morphine administration in rats and mice: effect of clonidine coadministration and sex-related differences. Turk J Med Sci 45: 1380-9.

Altarifi AA, Negus SS (2011) Some determinants of morphine effects on intracranial self-stimulation in rats: dose, pretreatment time, repeated treatment, and rate dependence. Behav Pharmacol 22: 663-73.

Ambrosio E, Goldberg SR, Elmer GI (1995) Behavior genetic investigation of the relationship between spontaneous locomotor activity and the acquisition of morphine self-administration behavior. Behav Pharmacol 6: 229-237.

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders: DSM-5. American Psychiatric Association, Washington, D.C.

Anker, J.J., Perry, J.L., Gliddon, L.A. and Carroll, M.E., 2009. Impulsivity predicts the escalation of cocaine self-administration in rats. Pharmacol. Biochem. and Behav., 93(3), pp.343-348.

Arrindell, W. A., & van der Ende, J., 1985. An empirical test of the utility of the observations- to-variables ratio in factor and components analysis. Appl. Psychol. Meas. 9, 165–178.

Back SE, Lawson KM, Singleton LM, Brady KT (2011) Characteristics and correlates of men and women with prescription opioid dependence. Addict Behav 36: 829-834.

Baker TB, Piper ME, McCarthy DE, Majeskie MR, Fiore MC (2004) Addiction motivation reformulated: an affective processing model of negative reinforcement. Psychol Rev 111: 33-51.

Badiani, A., Belin, D., Epstein, D., Calu, D., Shaham, Y., 2011. Opiate versus psychostimulant addiction: the differences do matter. Nature Reviews Neuroscience 12(11), 685-700.

Baker, T.B., Piper, M.E., McCarthy, D.E., Majeskie, M.R., Fiore, M.C., 2004 Addiction motivation reformulated: an affective processing model of negative reinforcement. Psychol. Rev. 111: 33-51.

Baldacchino, A., Balfour, D. J. K., & Matthews, K., 2015. Impulsivity and opioid drugs: differential effects of heroin, methadone and prescribed analgesic medication. Psychol. Med. 45(6), 1167-1179.

Ball KT, Jarsocrak H, Hyacinthe J, Lambert J, Lockowitz J, Schrock J (2015) Yohimbine reinstates extinguished 3, 4-methylenedioxymethamphetamine (MDMA; ecstasy) seeking in rats with prior exposure to chronic yohimbine. Behav brain research 294:1- 6.

Banks, M.L. and Negus, S.S., 2017. Insights from preclinical choice models on treating drug addiction. Trends in Pharmacol. Sci. 38(2), pp.181-194.

Banna, K. M., Back, S. E., Do, P., & See, R. E., 2010. Yohimbine stress potentiates conditioned cue-induced reinstatement of heroin-seeking in rats. Behav. Brain Res. 208(1), 144-148.

Bardo, M.T., Neisewander, J.L., Kelly, T.H., 2013. Individual differences and social influences on the neurobehavioral pharmacology of abused drugs. Pharmacol Rev 65(1), 255-290.

Barsy B, Mikics É, Barsvári B, Haller J (2011) The long-term impact of footshock stress on addiction-related behaviors in rats. Neuropharmacology 60(2-3):267-73.

Beauducel A, Herzberg PY. On the performance of maximum likelihood versus means and variance adjusted weighted least squares estimation in CFA. Structural Equation Modeling. 2006 Apr 28;13(2):186-203.

Becker JB, Koob GF (2016) Sex Differences in Animal Models: Focus on Addiction. Pharmacol Rev 68: 242-263.

Becker JB, McClellan ML, Reed BG (2017) Sex differences, gender and addiction. J Neurosci Res 95: 136-147.

Beckmann, J.S., Marusich, J.A., Gipson, C.D. and Bardo, M.T., 2011. Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. Behav brain res, 216(1), pp.159-165.

Belin D, Balado E, Piazza PV, Deroche-Gamonet V (2009) Pattern of intake and drug craving predict the development of cocaine addiction-like behavior in rats. Biol Psychiatry 65: 863-8.

Belin D, Belin-Rauscent A, Everitt BJ, Dalley JW (2016) In search of predictive endophenotypes in addiction: insights from preclinical research. Genes Brain Behav 15: 74-88.

Belin D, Berson N, Balado E, Piazza PV, Deroche-Gamonet V (2011) High-novelty-preference rats are predisposed to compulsive cocaine self-administration. Neuropsychopharmacology 36: 569-79.

Belin D, Deroche-Gamonet V (2012) Responses to novelty and vulnerability to cocaine addiction: contribution of a multi-symptomatic animal model. Cold Spring Harb Perspect Med 2.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320: 1352-5.

Bentzley, B.S., Fender, K.M. and Aston-Jones, G., 2013. The behavioral economics of drug self-administration: a review and new analytical approach for within-session procedures. Psychopharmacology, 226(1), pp.113-125.

Bickel, W.K., Marsch, L.A., Carroll, M.E., 2000. Deconstructing relative reinforcing efficacy and situating the measures of pharmacological reinforcement with behavioral economics: a theoretical proposal, Psychopharmacology (Berl.) 153(1), 44-56.

Bickel, W.K., DeGrandpre, R.J., Higgins, S.T., 1995. The behavioral economics of concurrent drug reinforcers: a review and reanalysis of drug self-administration research. Psychopharmacology (Berl) 118(3), 250-259.

Blanchard, R.J., Yudko, E.B., Rodgers, R.J. and Blanchard, D.C., 1993. Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. Behav. Brain Res. 58(1-2), pp.155-165.

Blanchard MM, Mendelsohn D, Stamp JA (2009) The HR/LR model: Further evidence as an animal model of sensation seeking. Neurosci Biobehav Rev 33: 1145-54.

Blanco C, Rafful C, Wall MM, Jin CJ, Kerridge B, Schwartz RP. The latent structure and predictors of non-medical prescription drug use and prescription drug use disorders: a national study. Drug and alcohol dependence. 2013 Dec 1;133(2):473-9.

Bruijnzeel AW, Marcinkiewcz C, Isaac S, Booth MM, Dennis DM, Gold MS (2007) The effects of buprenorphine on fentanyl withdrawal in rats. Psychopharmacology (Berl) 191: 931-41.

Budaev SV. Using principal components and factor analysis in animal behaviour research: caveats and guidelines. Ethology. 2010 May;116(5):472-80.

Cadoni, C., Simola, N., Espa, E., Fenu, S., Di Chiara, G., 2015. Strain dependence of adolescent Cannabis influence on heroin reward and mesolimbic dopamine transmission in adult Lewis and Fischer 344 rats. Addict Biol 20(1), 132-142.

Cahill K, Stead L, Lancaster T (2009) A preliminary benefit-risk assessment of varenicline in smoking cessation. Drug Saf 32: 119-35.

Cain, M.E., Saucier, D.A., Bardo, M.T., 2005. Novelty seeking and drug use: contribution of an animal model. Exp Clin Psychopharmacol 13(4), 367-375.

Carroll ME, Morgan AD, Anker JJ, Perry JL, Dess NK (2008) Selective breeding for differential saccharin intake as an animal model of drug abuse. Behav Pharmacol 19: 435-60.

Carroll ME, Morgan AD, Lynch WJ, Campbell UC, Dess NK (2002) Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. Psychopharmacology (Berl) 161: 304-13.

Cattell, R. B. (1978). The scientific use of factor analysis. New York: Plenum.

Center for Behavioral Health Statistics and Quality, 2013. The DAWN report: highlights of the 2011 Drug Abuse Warning Network (DAWN) findings on drug-related emergency department visits. Substance Abuse and Mental Health Services Administration, Rockville, MD.

Center for Behavioral Health Statistics and Quality, 2015. National survey on drug use and health: detailed tables. Substance Abuse and Mental Health Services Administration, Rockville, MD.

Center for Behavioral Health Statistics and Quality (2018) National survey on drug use and health: detailed tables. Substance Abuse and Mental Health Services Administration, Rockville, MD.

Center for Behavioral Health Statistics and Quality, 2020. *Results from the 2019 National Survey on Drug Use and Health: Detailed Tables.* Substance Abuse and Mental Health Services Administration, Rockville, MD.

Chang, J. Y., Janak, P. H. & Woodward, D. J. Comparison of mesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. J Neurosci. 18, 3098–3115 (1998)

Chaouloff, F., Kulikov, A., Sarrieau, A., Castanon, N., Mormède, P., 1995. Male Fischer 344 and Lewis rats display differences in locomotor reactivity, but not in anxiety-related behaviours: relationship with the hippocampal serotonergic system. Brain Res 693(1-2), 169-178.

Chappell AM, Weiner JL (2008) Relationship between ethanol's acute locomotor effects and ethanol self-administration in male Long-Evans rats. Alcohol Clin Exp Res 32: 2088-99.

Chase HW, Mackillop J, Hogarth L (2013) Isolating behavioural economic indices of demand in relation to nicotine dependence. Psychopharmacology (Berl) 226: 371-80.

Cheng, G.L., Liu, Y.P., Chan, C.C., So, K.F., Zeng, H., Lee, T.M., 2015. Neurobiological underpinnings of sensation seeking trait in heroin abusers. Eur Neuropsychopharmacol 25(11), 1968-1980.

Chester JA, Rausch EJ, June HL, Froehlich JC (2006) Decreased reward during acute alcohol withdrawal in rats selectively bred for low alcohol drinking. Alcohol 38: 165-72.

Childress, A. R., Hole, A. V., Ehrman, R. N., Robbins, S. J., McLellan, A. T., & O'Brien, C. P., 1993. Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res. Monogr. 137, 73-73.

Cippitelli A, Karlsson C, Shaw JL, Thorsell A, Gehlert DR, Heilig M (2010) Suppression of alcohol self-administration and reinstatement of alcohol seeking by melanin-concentrating hormone receptor 1 (MCH1-R) antagonism in Wistar rats. Psychopharmacology 211(4):367-75.

Cohen, A., Young, R. W., Velazquez, M. A., Groysman, M., Noorbehesht, K., Ben-Shahar, O. M., & Ettenberg, A., 2009. Anxiolytic effects of nicotine in a rodent test of approach–avoidance conflict. Psychopharmacology 204(3), 541-549.

Conrod, P. J., Pihl, R. O., Stewart, S. H., & Dongier, M., 2000. Validation of a system of classifying female substance abusers on the basis of personality and motivational risk factors for substance abuse. Psychol of Addict Behav 14(3), 243-256.

Cooperman AW, Waller NG. Heywood you go away! Examining causes, effects, and treatments for Heywood cases in exploratory factor analysis. *In press*.

Dellu, F., Piazza, P., Mayo, W., Le Moal, M., Simon, H., 1996. Novelty-seeking in rats biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology 34(3), 136-145.

Deminiere JM, Piazza PV, Le Moal M, Simon H (1989) Experimental approach to individual vulnerability to psychostimulant addiction. Neurosci Biobehav Rev 13: 141-7.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V., 2004. Evidence for addiction-like behavior in the rat. Science. 305(5686), 1014-1017.

Dess NK, O'Neill P, Chapman CD (2005) Ethanol withdrawal and proclivity are inversely related in rats selectively bred for differential saccharin intake. Alcohol 37: 9-22.

de Wit, H., 1996. Priming effects with drugs and other reinforcers. Experimental and Clinical Psychopharmacology. 4(1), 5.

Dickson, P.E., Ndukum, J., Wilcox, T., Clark, J., Roy, B., Zhang, L., Li, Y., Lin, D.T. and Chesler, E.J., 2015. Association of novelty-related behaviors and intravenous cocaine self-administration in Diversity Outbred mice. Psychopharmacology, 232(6), pp.1011-1024.

Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer AN, DeVries TJ (2008) Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. Biol Psychiatry 63(3): 301-308.

DiFranza JR, Savageau JA, Fletcher K, Ockene JK, Rigotti NA, McNeill AD, Coleman M, Wood C (2004) Recollections and repercussions of the first inhaled cigarette. Addict Behav 29: 261-72.

Dilleen R, Pelloux Y, Mar AC, Molander A, Robbins TW, Everitt BJ, Dalley JW, Belin D (2012) High anxiety is a predisposing endophenotype for loss of control over cocaine, but not heroin, self-administration in rats. Psychopharmacology (Berl) 222: 89-97.

Edwards, S., & Koob, G. F., 2013. Escalation of drug self-administration as a hallmark of persistent addiction liability. Behav. Pharmacol. 24(0).

Engelmann JM, Radke AK, Gewirtz JC (2009) Potentiated startle as a measure of the negative affective consequences of repeated exposure to nicotine in rats. Psychopharmacology (Berl) 207: 13-25.

Ersche, K. D., Turton, A. J., Pradhan, S., Bullmore, E. T., & Robbins, T. W., 2010. Drug addiction endophenotypes: impulsive versus sensation-seeking personality traits. Biol. Psychiatry. 68(8), 770-773.

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y., 2006. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology. 189(1), 1-16.

Ettenberg, A., Pettit, H. O., Bloom, F. E., & Koob, G. F., 1982. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology. 78(3), 204-209.

Everitt, B. S., 1975. Multivariate analysis: The need for data, and other problems. British J. Psychiatry. 126, 237–240.

Facchinetti, F., Volpe, A., Farci, G., Petraglia, F., Porro, C.A., Barbieri, G., Cioni, A., Balestrieri, A. & Genazzani, A.R., 1985. Hypothalamus-pituitary-adrenal axis of heroin addicts. Drug Alcohol Depend. 15(4), 361-366.

Fareed, A., Eilender, P., Haber, M., Bremner, J., Whitfield, N., & Drexler, K., 2013. Comorbid posttraumatic stress disorder and opiate addiction: a literature review. J. Addict. Dis. 32(2), 168-179.

Flagel, S.B., Akil, H. and Robinson, T.E., 2009. Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology, 56, pp.139-148.

Flagel, S.B., Chaudhury, S., Waselus, M., Kelly, R., Sewani, S., Clinton, S.M., Thompson, R.C., Watson, S.J. and Akil, H., 2016. Genetic background and epigenetic modifications in the core of the nucleus accumbens predict addiction-like behavior in a rat model. Proceedings of the National Academy of Sciences, 113(20), pp.E2861-E2870.

Fowler C.D., Kenny P.J. (2014) Nicotine aversion: Neurobiological mechanisms and relevance to tobacco dependence vulnerability. Neuropharmacology 76 Pt B: 533-44.

Franques, P., Auriacombe, M., Piquemal, E., Verger, M., Brisseau-Gimenez, S., Grabot, D., Tignol, J., 2003. Sensation seeking as a common factor in opioid dependent subjects and high risk sport practicing subjects. A cross sectional study. Drug Alcohol Depend 69(2), 121-126.

Funk C.K., O'Dell L.E., Crawford E.F., Koob G.F. (2006) Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. J Neurosci 26: 11324-32.

George O, Koob G.F. Individual differences in prefrontal cortex function and the transition from drug use to drug dependence. Neuroscience & Biobehavioral Reviews. 2010 Nov 1;35(2):232-47.

Geyer, M.A. and Markou, A., 1995. Animal models of psychiatric disorders. Psychopharmacology: the fourth generation of progress, pp.787-798.

Gileta, A. F., Fitzpatrick, C. J., Chitre, A. S., Pierre, C. L. S., Joyce, E. V., Maguire, R. J., ... & Robinson, T. E., 2018. Genetic characterization of outbred Sprague Dawley rats and utility for genome-wide association studies. bioRxiv, 412924.

Gillespie NA, Neale MC, Prescott CA, Aggen SH, Kendler KS. Factor and item-response analysis DSM-IV criteria for abuse of and dependence on cannabis, cocaine, hallucinogens, sedatives, stimulants and opioids. Addiction. 2007 Jun;102(6):920-30.

Goeders, N. E. 2011. The Hypothalamic-Pituitary-Adrenal Axis and Addiction, in: Al'Absi, M. ed., 2011. Stress and addiction: Biological and psychological mechanisms. Elsevier.

Goldberg L.R., Velicer W.F. Principles of exploratory factor analysis. Differentiating normal and abnormal personality. 2006;2:209-337.

Gorsuch, R. L., 1983. Factor analysis (2nd ed.). Hillsdale, NJ: Erlbaum.

Grebenstein P, Burroughs D, Zhang Y, LeSage MG (2013) Sex differences in nicotine self-administration in rats during progressive unit dose reduction: implications for nicotine regulation policy. Pharmacol Biochem Behav 114-115: 70-81.

Grebenstein, P.E., Burroughs, D., Roiko, S.A., Pentel, P.R., LeSage, M.G., 2015. Predictors of the nicotine reinforcement threshold, compensation, and elasticity of demand in a rodent model of nicotine reduction policy. Drug Alcohol Depend 151, 181-193.

Greenwald, M.K., Hursh, S.R., 2006. Behavioral economic analysis of opioid consumption in heroin-dependent individuals: effects of unit price and pre-session drug supply. Drug Alcohol Depend 85(1), 35-48.

Guilford, J. P., 1954. Psychometric methods (2nd ed.). New York: McGraw-Hill.

Hansson A.C., Cippitelli A, Sommer W.H., Fedeli A., Björk K., Soverchia L., Terasmaa A., Massi M., Heilig M., Ciccocioppo R. Variation at the rat Crhr1 locus and sensitivity to relapse into alcohol seeking induced by environmental stress. Proceedings of the National Academy of Sciences. 2006 Oct 10;103(41):15236-41.

Harris A.C., Gewirtz J.C. (2004) Elevated startle during withdrawal from acute morphine: a model of opiate withdrawal and anxiety. Psychopharmacology (Berl) 171: 140-7.

Harris A.C., Gewirtz J.C. (2005) Acute opioid dependence: characterizing the early adaptations underlying drug withdrawal. Psychopharmacology (Berl) 178: 353-66.

Harris A.C., Hanes S.L., Gewirtz J.C. (2004) Potentiated startle and hyperalgesia during withdrawal from acute morphine: effects of multiple opiate exposures. Psychopharmacology (Berl) 176: 266-73.

Harris A.C., Manbeck K.E., Schmidt C.E., Shelley D. (2013) Mecamylamine elicits withdrawal-like signs in rats following a single dose of nicotine. Psychopharmacology (Berl) 225: 291-302.

Harris A.C., Pentel P.R., Burroughs D., Staley M.D., Lesage M.G. (2011) A lack of association between severity of nicotine withdrawal and individual differences in compensatory nicotine self-administration in rats. Psychopharmacology (Berl) 217: 153-66.

Harris A.C., Tally L., Muelken P., Banal A., Schmidt C.E., Cao Q., LeSage M.G. (2015) Effects of nicotine and minor tobacco alkaloids on intracranial-self-stimulation in rats. Drug Alcohol Depend 153: 330-4.

Harty, S.C., Whaley, J.E., Halperin, J.M. and Ranaldi, R., 2011. Impulsive choice, as measured in a delay discounting paradigm, remains stable after chronic heroin administration. Pharmacol. Biochem. Behav. 98(3), pp.337-340.

Hastie T, Tibshirani R, Friedman J. The elements of statistical learning: data mining, inference, and prediction. Springer Science & Business Media; 2009 Aug 26.

Heyman, G. M., 2009. Addiction: A disorder of choice. Harvard University Press.

Hernandez-Avila C.A., Rounsaville B.J., Kranzler H.R. Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. Drug Alcohol Depend. 2004;74(3):265-272.

Heywood H.B. On finite sequences of real numbers. Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character. 1931 Dec 2;134(824):486-501.

Hicks B.M., Schalet B.D., Malone S.M., Iacono W.G., McGue M. Psychometric and genetic architecture of substance use disorder and behavioral disinhibition measures for gene association studies. Behavior genetics. 2011 Jul;41(4):459-75.

Hittner, J. B., & Swickert, R., 2006. Sensation seeking and alcohol use: A meta-analytic review. Addict behav, 31(8), 1383-1401.

Hodos, W., 1961. Progressive ratio as a measure of reward strength. Science. 134(3483), 943-944.

Holtz N.A., Radke A.K., Zlebnik N.E., Harris A.C., Carroll M.E. (2015) Intracranial self-stimulation reward thresholds during morphine withdrawal in rats bred for high (HiS) and low (LoS) saccharin intake. Brain Res 1602: 119-26.

Hughson, A.R., Horvath, A.P., Holl, K., Palmer, A.A., Woods, L.C.S., Robinson, T.E. & Flagel, S.B., 2019. Incentive salience attribution, “sensation-seeking” and

"novelty-seeking" are independent traits in a large sample of male and female heterogeneous stock rats. Sci. Rep. 9(1), 1-16.

Hu L.T, Bentler P.M. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural equation modeling: a multidisciplinary journal. 1999 Jan 1;6(1):1-55.

Hur, Y. M., & Bouchard, T. J., 1997. The genetic correlation between impulsivity and sensation seeking traits. Behav. Genet. 27(5), 455-463.

Hursh, S.R., 1991. Behavioral economics of drug self-administration and drug abuse policy. J Exp Anal Behav 56(2), 377-393.

Hursh S.R, Silberberg A. (2008) Economic demand and essential value. Psychol Rev 115: 186-98.

Husky M.M., Mazure C.M., Paliwal P, McKee S.A. Gender differences in the comorbidity of smoking behavior and major depression. Drug Alcohol Depend. 2008;93(1-2):176- 179.

Ibáñez M.I, Ávila C, Ruipérez MA, Moro M, Ortet G. Temperamental traits in mice (I): Factor structure. Personality and Individual Differences. 2007 Jul 1;43(2):255-65.

Igari M, Alexander J.C., Ji Y., Qi X., Papke R.L., Bruijnzeel A.W. (2014) Varenicline a nd cytisine diminish the dysphoric-like state associated with spontaneous nicotine withdrawal in rats. Neuropsychopharmacology 39: 455-65.

Jacobucci, R., Grimm, K. J., & McArdle, J. J., 2016. Regularized structural equation modeling. Structural Equation Modeling: A Multidisciplinary Journal. 23(4), 555-566.

Jones CM, Einstein EB, Compton WM (2018) Changes in Synthetic Opioid Involvement in Drug Overdose Deaths in the United States, 2010-2016. JAMA 319: 1819-1821.

Jung, S., & Takane, Y., 2007. Regularized common factor analysis. New Trends in Psychometrics. 141-149.

Jung, S., & Lee, S., 2011. Exploratory factor analysis for small samples. Behav. Res. Methods. 43(3), 701-709.

Kabbaj, M., 2006. Individual differences in vulnerability to drug abuse: the high responders/low responders model. CNS Neurol Disord Drug Targets 5(5), 513-520.

Katz, J. L., 1990. Models of relative reinforcing efficacy of drugs and their predictive utility. Behav. Pharmacol. 1(4), 283-301.

Khantzian, E. J., 1987. The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. In *The cocaine crisis*, 65-74. Springer, Boston, MA.

Kenny PJ, Chen SA, Kitamura O, Markou A, Koob GF (2006) Conditioned withdrawal drives heroin consumption and decreases reward sensitivity. J Neurosci 26: 5894-900.

Kenny PJ, Koob GF, Markou A (2003) Conditioned facilitation of brain reward function after repeated cocaine administration. Behav Neurosci 117: 1103-7.

Kiluk BD, Yip SW, DeVito EE, Carroll KM, Sofuoglu M (2019) Anhedonia as a Key Clinical Feature in the Maintenance and Treatment of Opioid Use Disorder. Clinical Psychological Science: 2167702619855659.

Kitamura, O., Wee, S., Specio, S. E., Koob, G. F., & Pulvirenti, L., 2006. Escalation of methamphetamine self-administration in rats: a dose–effect function. Psychopharmacology. 186(1), 48-53.

Kline, P., 1979. Psychometrics and psychology. London: Academic Press

Koffarnus MN, Franck CT, Stein JS, Bickel WK (2015) A modified exponential behavioral economic demand model to better describe consumption data. Exp Clin Psychopharmacol 23: 504-12.

Kolenikov S, Bollen KA. Testing negative error variances: Is a Heywood case a symptom of misspecification?. Sociological Methods & Research. 2012 Feb;41(1):124-67.

Koob, G. F. (2013). Addiction is a reward deficit and stress surfeit disorder. Front. Psychiatry, 4, 72.

Koob G.F., Le Moal M. (2005) Plasticity of reward neurocircuitry and the 'dark side' of drug addiction. Nat Neurosci 8: 1442-4.

Koob G.F., Le Moal M. (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278: 52-8.

Kosten, T.A., Ball, S.A., Rounsaville, B.J., 1994. A sibling study of sensation seeking and opiate addiction. J Nerv Ment Dis 182(5), 284-289.

Kreek, M. J., Nielsen, D. A., Butelman, E. R., & LaForge, K. S., 2005. Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat. Neurosci. 8(11), 1450.

Krueger, R. F., Hicks, B. M., Patrick, C. J., Carlson, S. R., Iacono, W. G., & McGue, M., 2002. Etiologic connections among substance dependence, antisocial behavior and personality: Modeling the externalizing spectrum. J. Abnorm. Psychol. 111(3), 411.

Kurth-Nelson, Z. & Redish, A.D. 2010. A reinforcement learning model of precommitment in decision making. Front. Behav. Neurosci., 4, 184.

Le AD, Harding S, Juzytsch W, Funk D, Shaham Y (2005) Role of alpha-2 adrenoceptors in stress-induced reinstatement of alcohol seeking and alcohol self-administration in rats. Psychopharmacology 179(2):366-73.

Le AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y (1998) Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. Psychopharmacol 135(2): 169-74.

Lejuez, C. W., Zvolensky, M. J., Daughters, S. B., Bornovalova, M. A., Paulson, A., Tull, M. T., ... & Otto, M. W., 2008. Anxiety sensitivity: A unique predictor of dropout among inner-city heroin and crack/cocaine users in residential substance use treatment. Behav Res Ther 46(7), 811-818.

Lennox R, Dennis ML, Scott CK, Funk R. Combining psychometric and biometric measures of substance use. Drug and Alcohol Dependence. 2006 Jun 28;83(2):95-103.

Leri, F., Tremblay, A., Sorge, R. E., & Stewart, J., 2004. Methadone maintenance reduces heroin-and cocaine-induced relapse without affecting stress-induced relapse in a rodent model of poly-drug use. Neuropsychopharmacol. 29(7), 1312.

LeSage, M.G., Staley, M., Muelken, P., Smethells, J.R., Stepanov, I., Vogel, R.I., Pentel,

P.R., Harris, A.C., 2016. Abuse liability assessment of an e-cigarette refill liquid using intracranial self-stimulation and self-administration models in rats. Drug Alcohol Depend 168, 76-88.

LeSage M.G., Staley M., Muelken P., Smethells J.R., Stepanov I., Vogel R.I., Pentel P.R., Harris A.C. (2016) Abuse liability assessment of an e-cigarette refill liquid using intracranial self-stimulation and self-administration models in rats. Drug Alcohol Depend 168: 76-88.

Li, S.X., Li, J., Epstein, D.H., Zhang, X.Y., Kosten, T.R. and Lu, L., 2008. Serum cortisol secretion during heroin abstinence is elevated only nocturnally. Am. J. Drug Alcohol Abuse, 34(3), pp.321-328.

Liu J, Schulteis G (2004) Brain reward deficits accompany naloxone-precipitated withdrawal from acute opioid dependence. Pharmacol Biochem Behav 79: 101-8.

Liu X, Weiss F (2002) Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. J of Neurosci 22(18): 7856-61.

Luczak, S.E., Glatt, S.J. and Wall, T.L., 2009. Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. Psychol Bull. 132, pp.607-21.

Lynch WJ, Roth ME, Carroll ME. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology (Berl.). 2002;164(2):121-137. (1997).

Lynskey, M. T., & Agrawal, A., 2007. Psychometric properties of DSM assessments of illicit drug abuse and dependence: results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). Psychol. Med. 37(9), 1345-1355.

MacCallum RC, Austin JT. Applications of structural equation modeling in psychological research. Annual review of psychology. 2000 Feb;51(1):201-26.

Mantsch, J.R., Ho, A., Schlussman, S.D., Kreek, M.J., 2001. Predictable individual differences in the initiation of cocaine self-administration by rats under extended-access conditions are dose-dependent. Psychopharmacology (Berl) 157(1), 31-39.

Marino, E.N., Rosen, K.D., Gutierrez, A., Eckmann, M., Ramamurthy, S., Potter, J.S., 2013. Impulsivity but not sensation seeking is associated with opioid analgesic misuse risk in patients with chronic pain. Addict Behav 38(5), 2154-2157.

Markou A, Koob GF (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology 4: 17-26.

Markou A, Koob GF (1992) Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. Physiol Behav 51: 111-9.

Martins, S. S., Fenton, M. C., Keyes, K. M., Blanco, C., Zhu, H., & Storr, C. L., 2012. Mood and anxiety disorders and their association with non-medical prescription opioid use and prescription opioid-use disorder: longitudinal evidence from the National Epidemiologic Study on Alcohol and Related Conditions. Psychol Med 42(6), 1261-1272.

Marusich, J.A., Darna, M., Charnigo, R.J., Dwoskin, L.P. and Bardo, M.T., 2011. A multivariate assessment of individual differences in sensation seeking and

impulsivity as predictors of amphetamine self-administration and prefrontal dopamine function in rats. Exp. Clin. Psychopharmacol, 19(4), p.275.

Maydeu-Olivares A, Shi D, Rosseel Y. Assessing fit in structural equation models: A Monte-Carlo evaluation of RMSEA versus SRMR confidence intervals and tests of close fit. Structural Equation Modeling: A Multidisciplinary Journal. 2018 May 4;25(3):389-402.

McHugh, R. K., Weiss, R. D., Cornelius, M., Martel, M. O., Jamison, R. N., & Edwards, R. R., 2016. Distress intolerance and prescription opioid misuse among patients with chronic pain. J. Pain. 17(7), 806-814.

McNamara R, Dalley JW, Robbins TW, Everitt BJ, Belin D (2010) Trait-like impulsivity does not predict escalation of heroin self-administration in the rat. Psychopharmacol (Berl) 212: 453-64.

Mendrek A, Blaha CD, Phillips AG (1998) Pre-exposure of rats to amphetamine sensitizes self-administration of this drug under a progressive ratio schedule.

Meyer, A.C., Rahman, S., Charnigo, R.J., Dwoskin, L.P., Crabbe, J.C., Bardo, M.T., 2010. Genetics of novelty seeking, amphetamine self-administration and reinstatement using inbred rats. Genes Brain Behav 9(7), 790-798.

Mitchell, J.M., Cunningham, C.L., Mark, G.P., 2005. Locomotor activity predicts acquisition of self-administration behavior but not cocaine intake. Behav Neurosci 119(2), 464-472.

Moazen, P., Azizi, H., Salmanzadeh, H., & Semnanian, S., 2018. Adolescent morphine exposure induces immediate and long-term increases in impulsive behavior. Psychopharmacology. 235(12), 3423-3434.

Monga, N., Rehm, J., Fischer, B., Brissette, S., Bruneau, J., El-Guebaly, N., ... & Fallu, J. S., 2007. Using latent class analysis (LCA) to analyze patterns of drug use in a population of illegal opioid users. Drug Alcohol Depend. 88(1), 1-8.

Neale MC, Kendler KS. Models of comorbidity for multifactorial disorders. American journal of human genetics. 1995 Oct;57(4):935.

Nielsen, D. A., Ho, A., Bahl, A., Varma, P., Kellogg, S., Borg, L., & Kreek, M. J., 2012. Former heroin addicts with or without a history of cocaine dependence are more impulsive than controls. Drug Alcohol Depend 124(1), 113-120.

Nishida, K.S., Park, T.Y., Lee, B.H., Ursano, R.J., Choi, K.H., 2016. Individual differences in initial morphine sensitivity as a predictor for the development of opiate addiction in rats. Behav Brain Res 313, 315-323.

Norton, G., 2001. Substance use/abuse and anxiety sensitivity: What are the relationships? Addict Behav 26(6), 935-946.

Nestler EJ, Carlezon WA, Jr. (2006) The mesolimbic dopamine reward circuit in depression. Biol Psychiatry 59: 1151-9.

Nishida KS, Park TY, Lee BH, Ursano RJ, Choi KH (2016) Individual differences in initial morphine sensitivity as a predictor for the development of opiate addiction in rats. Behav Brain Res 313: 315-323.

O'Connor, E. C., Chapman, K., Butler, P., & Mead, A. N., 2011. The predictive validity of the rat self-administration model for abuse liability. Neurosci. Biobehav. Rev. 35(3), 912-938.

O'Dell LE (2009) A psychobiological framework of the substrates that mediate nicotine use during adolescence. Neuropharmacology 56 Suppl 1: 263-78.

O'Dell LE, Bruijnzeel AW, Smith RT, Parsons LH, Merves ML, Goldberger BA, Richardson HN, Koob GF, Markou A (2006) Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. Psychopharmacology (Berl) 186: 612-9.

O'Loughlin J, DiFranza J, Tyndale RF, Meshefedjian G, McMillan-Davey E, Clarke PB, Hanley J, Paradis G (2003) Nicotine-dependence symptoms are associated with smoking frequency in adolescents. Am J Prev Med 25: 219-25.

Palmer RH, Brick L, Nugent NR, Bidwell LC, McGeary JE, Knopik VS, Keller MC. Examining the role of common genetic variants on alcohol, tobacco, cannabis and illicit drug dependence: genetics of vulnerability to drug dependence. Addiction. 2015 Mar;110(3):530-7.

Park, T.Y., Nishida, K.S., Wilson, C.M., Jaiswal, S., Scott, J., Hoy, A.R., Selwyn, R.G., Dardzinski, B.J., Choi, K.H., 2017. Effects of isoflurane anesthesia and intravenous morphine self-administration on regional glucose metabolism ([(18) F]FDG-PET) of male Sprague-Dawley rats. Eur J Neurosci 45(7), 922-931.

Parker, C. C., Chen, H., Flagel, S. B., Geurts, A. M., Richards, J. B., Robinson, T. E., ... & Palmer, A. A., 2014. Rats are the smart choice: rationale for a renewed focus on rats in behavioral genetics. Neuropharmacol. 76, 250-258.

Pawlak CR, Ho YJ, Schwarting RK (2008) Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci Biobehav Rev 32: 1544-68.

Pederson AA (2009) Reliability and validity of a demand curve measure of alcohol reinforcement. Exp Clin Psychopharmacol 17: 396-404.

Pelloux, Y., Costentin, J., Duterte-Boucher, D., 2009. Anxiety increases the place conditioning induced by cocaine in rats. Behav Brain Res 197(2), 311–316

Perry, J.L., Larson, E.B., German, J.P., Madden, G.J. and Carroll, M.E., 2005. Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. Psychopharmacology, 178(2-3), pp.193-201.

Pettit, H. O., Ettenberg, A., Bloom, F. E., & Koob, G. F., 1984. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology. 84(2), 167-173.

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245: 1511-3.

Piazza, P. V., & Le Moal, M., 1996. Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu. Rev. Pharmacol. Toxicol. 36(1), 359-378.

Piazza, P.V., Deroche-Gamonent, V., Rouge-Pont, F., Le Moal, M., 2000. Vertical shifts in self administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20(11), 4226-4232.

Pizzimenti CL, Navis TM, Lattal KM (2017) Persistent effects of acute stress on fear and drug-seeking in a novel model of the comorbidity between post-traumatic stress disorder and addiction. Learning & Memory 24(9):422-31.

Prut, L., & Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J of Pharmacol 463(1), 3-33.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Radke AK, Zlebnik NE, Carroll ME (2015) Cocaine withdrawal in rats selectively bred for low (LoS) versus high (HiS) saccharin intake. Pharmacol Biochem Behav 129: 51-5.

Raleigh, M.D., Pentel, P.R., LeSage, M.G., 2014. Pharmacokinetic correlates of the effects of a heroin vaccine on heroin self-administration in rats. PLoS One 9(12), e115696.

Redonnet, B., Chollet, A., Fombonne, E., Bowes, L., & Melchior, M., 2012. Tobacco, alcohol, cannabis and other illegal drug use among young adults: The socioeconomic context. Drug Alcohol Depend. 121(3), 231-239.

Richardson, N. R., & Roberts, D. C., 1996. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods. 66(1), 1-11.

Rogers, A. H., Bakhshaie, J., Lam, H., Langdon, K. J., Ditre, J. W., & Zvolensky, M. J., 2018. Pain-related anxiety and opioid misuse in a racially/ethnically diverse young adult sample with moderate/severe pain. Cogn. Behav. Ther. 47(5), 372-382.

Rosseel Y. Lavaan: An R package for structural equation modeling and more. Version 0.5–12 (BETA). Journal of statistical software. 2012 Dec 19;48(2):1-36.

Rothwell PE, Gewirtz JC, Thomas MJ (2010) Episodic withdrawal promotes psychomotor sensitization to morphine. Neuropsychopharmacology 35: 2579-89.

Rousseeuw, P. J., & Driessen, K. V. (1999). A fast algorithm for the minimum covariance determinant estimator. Technometrics, 41(3), 212-223.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA

Sartor CE, Lessov-Schlaggar CN, Scherrer JF, Bucholz KK, Madden PA, Pergadia ML, Grant JD, Jacob T, Xian H (2010) Initial response to cigarettes predicts rate of progression to regular smoking: findings from an offspring-of-twins design. Addict Behav 35: 771-8.

Schippers, M. C., Binnekade, R., Schoffelmeer, A. N., Pattij, T., & De Vries, T. J., 2012. Unidirectional relationship between heroin self-administration and impulsive decision-making in rats. Psychopharmacology. 219(2), 443-452.

Schmitt, T.A., 2011. Current methodological considerations in exploratory and confirmatory factor analysis. Journal of Psychoeducational Assessment, 29(4), pp.304-321.

Schuckit MA, Smith TL, Kalmijn J (2004) The search for genes contributing to the low level of response to alcohol: patterns of findings across studies. Alcohol Clin Exp Res 28: 1449-58.

Schulteis G, Markou A, Gold LH, Stinus L, Koob GF (1994) Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. J Pharmacol Exp Ther 271: 1391-8.

Schulteis G, Morse AC, Liu J (2004) Conditioning processes contribute to severity of naloxone-precipitated withdrawal from acute opioid dependence. Psychopharmacology (Berl) 175: 463-72.

Shaham, Y., 1993. Immobilization stress-induced oral opioid self-administration and withdrawal in rats: role of conditioning factors and the effect of stress on "relapse" to opioid drugs. Psychopharmacology. 111(4), 477-485.

Shaham, Y. & Stewart, J., 1994. Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. Psychopharmacology, 114(3), 523-527.

Shekhar, A., McCann, U., Meaney, M., Blanchard, D., Davis, M., Frey, K., Liberzon, I., Overall, K., Shear, M., Tecott, L. and Winsky, L., 2001. Summary of a National Institute of Mental Health workshop: developing animal models of anxiety disorders. Psychopharmacology. 157(4), pp.327-339.

Shepard JD, Bossert JM, Liu SY, Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biol Psychiatry 55: 1082-9.

Shoaib M, Schindler CW, Goldberg SR (1997) Nicotine self-administration in rats: strain and nicotine pre-exposure effects on acquisition. Psychopharmacology (Berl) 129: 35-43.

Sinha, R., 2001. How does stress increase risk of drug abuse and relapse?. Psychopharmacology. 158(4), 343-359.

Smith, M.A. & Pitts, E.G. 2012. Wheel running decreases the positive reinforcing effects of heroin. Pharmacol Reports, 64(4), 960-964.

Smith, M.A., 2020. Nonhuman animal models of substance use disorders: Translational value and utility to basic science. Drug Alcohol Depend. 206, 107733.

Smith, T.T., Schaff, M.B., Rupprecht, L.E., Schassburger, R.L., Buffalari, D.M., Murphy, S.E., Sved, A.F., Donny, E.C., 2015. Effects of MAO inhibition and a combination of minor alkaloids, beta-carbolines, and acetaldehyde on nicotine self-administration in adult male rats. Drug Alcohol Depend 155, 243-252.

Sorge, R. E., Rajabi, H., & Stewart, J., 2005. Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement. Neuropsychopharmacol. 30(9), 1681.

Stafford, N. P., Kazan, T. N., Donovan, C. M., Hart, E. E., Drugan, R. C., & Charntikov, S., 2019. Individual vulnerability to stress is associated with increased demand for intravenous heroin self-administration in rats. Front. Behav. Neurosci. 13.

Strickland, J. C., Lile, J. A., & Stoops, W. W., 2019. Evaluating non-medical prescription opioid demand using commodity purchase tasks: test-retest reliability and incremental validity. Psychopharmacology. 1-12.

Suto, N., Austin, J.D., Vezina, P., 2001. Locomotor response to novelty predicts a rat's propensity to self-administer nicotine. Psychopharmacology (Berl) 158(2), 175-180.

Swann, A. C., Lijffijt, M., Lane, S. D., Steinberg, J. L., & Moeller, F. G., 2009. Trait impulsivity and response inhibition in antisocial personality disorder. J. Psychiatr. Res. 43(12), 1057-1063.

Tibshirani R. Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society: Series B (Methodological). 1996 Jan;58(1):267-88.

Townsend, E. A., Negus, S. S., Caine, S. B., Thomsen, M., & Banks, M. L., 2019. Sex differences in opioid reinforcement under a fentanyl vs. food choice procedure in rats. Neuropsychopharmacol. 44, 2022–2029.

Tran-Nguyen LT, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL (1998) Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacol 19(1):48-59.

Treit, D., & Fundytus, M., 1988. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol Biochem Behav 31(4), 959-962.

Treutlein J, Kissling C, Frank J, Wiemann S, Dong L, Depner M, Saam C, Lascorz J, Soyka M, Preuss UW, Rujescu D. Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. Molecular psychiatry. 2006 Jun;11(6):594-602.

Tsuang, M.T., Lyons, M.J., Meyer, J.M., Doyle, T., Eisen, S.A., Goldberg, J., True, W., Lin, N., Toomey, R. and Eaves, L., 1998. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. Arch Gen Psychiatry, 55(11), pp.967-972.

Turner, C.A., Flagel, S.B., Clinton, S.M., Akil, H., Watson, S.J., 2008. Cocaine interacts with the novelty-seeking trait to modulate FGFR1 gene expression in the rat. Neurosci Lett 446(2-3), 105-107.

Vassoler FM, Oliver DJ, Wyse C, Blau A, Shtutman M, Turner JR, Byrnes EM (2017) Transgenerational attenuation of opioid self-administration as a consequence of adolescent morphine exposure. Neuropharmacolo 113: 271-280.

Venables WN, Ripley BD (2002). Modern Applied Statistics with S, Fourth edition. Springer, New York. ISBN 0-387-95457-0, https://www.stats.ox.ac.uk/pub/MASS4/

Venniro, M., & Shaham, Y. 2020. An operant social self-administration and choice model in rats. Nature Protocols, 15(4), 1542-1559.

Vest, N., Reynolds, C. J., & Tragesser, S. L., 2016. Impulsivity and risk for prescription opioid misuse in a chronic pain patient sample. Addict Behav, 60, 184-190.

Vowles, K. E., McEntee, M. L., Julnes, P. S., Frohe, T., Ney, J. P., & van der Goes, D. N., 2015. Rates of opioid misuse, abuse, and addiction in chronic pain: a systematic review and data synthesis. Pain. 156(4), 569-576.

Wall PM, Messier C. Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. Behavioural Brain Research. 2000 Sep 1;114(1-2):199-212.

Waller NG. fungible: Psychometric Functions from the Waller Lab.. version 1.95.4.8. 2020

Walker, Q.D., Schramm-Sapyta, N.L., Caster, J.M., Waller, S.T., Brooks, M.P., Kuhn,

C.M., 2009. Novelty induced locomotion is positively associated with cocaine ingestion in adolescent rats; anxiety is correlated in adults. Pharmacol Biochem Behav 91(3), 398–408.

Wang SC, Chen YC, Lee CH, Cheng CM (2019) Opioid Addiction, Genetic Susceptibility, and Medical Treatments: A Review. Int J Mol Sci 20.

Whiteside, S. P., & Lynam, D. R., 2001. The five factor model and impulsivity: Using a structural model of personality to understand impulsivity. Personal. Individual Differ. 30(4), 669-689.

de Winter JC, Dodou D. Factor recovery by principal axis factoring and maximum likelihood factor analysis as a function of factor pattern and sample size. Journal of Applied Statistics. 2012 Apr 1;39(4):695-710.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5: 483-94.

Worley MJ, Shoptaw SJ, Bickel WK, Ling W (2015) Using behavioral economics to predict opioid use during prescription opioid dependence treatment. Drug Alcohol Depend 148: 62-8.

Yap JJ, Miczek KA (2008) Stress and Rodent Models of Drug Addiction: Role of VTA-Accumbens-PFC-Amygdala Circuit. Drug Discov Today Dis Models 5: 259-270.

Zhou Y, Cui CL, Schlussman SD, Choi JC, Ho A, Han JS, Kreek MJ (2008) Effects of cocaine place conditioning, chronic escalating-dose "binge" pattern cocaine administration and acute withdrawal on orexin/hypocretin and preprodynorphin gene expressions in lateral hypothalamus of Fischer and Sprague-Dawley rats. Neuroscience 153: 1225-34.

Zuckerman, M., (1994). Behavioral expressions and biosocial bases of sensation seeking. Cambridge University Press.

Appendix: Supplemental Materials (Study 2)

ICSS

Apparatus

Rats were tested in operant conditioning chambers (29×26×33 cm; Med Associates, St. Albans, VT, USA) placed inside sound-attenuating cubicles. A 5-cm-wide metal wheel manipulandum was fixed to the front wall. Brain stimulation was administered with constant current stimulators (model #PHM-152, Med Associates). Rats were connected to the stimulation circuit through bipolar leads (Plastics One, Roanoke, VA, USA) attached to gold-contact swivel commutators (Plastics One). MED-PC IV software was used to control stimulation parameters and for data collection.

Surgery

Animals were anesthetized with ketamine (75 mg/kg, i.m.) and dexmedetomidine (0.5 mg, i.m.) and implanted with a bipolar stainless-steel electrode (Plastics One) in the medial forebrain bundle at the level of the lateral hypothalamus as described in Roiko et al. (2009). Animals were allowed to recover for at least 1 week prior to ICSS training. During the first 2 days of recovery, all animals received injections of the antibiotic ceftriaxone (5.25 mg, i.m.) and the analgesic buprenorphine (0.1 mg/kg, s.c.).

General Testing Procedures

ICSS. Each trial was initiated with presentation of a non-contingent stimulus (0.1-ms cathodal square wave pulses at a frequency of 100 Hz for 500 ms) followed by a 7.5-s window, during which a positive response on the wheel manipulandum produced a second contingent stimulation identical to the first. Lack of responding during the 7.5-s window was considered a negative response. Each positive or negative response was followed by a

variable inter-trial interval averaging 10 s (range, 7.5–12.5 s), during which time additional responses delayed the onset of the subsequent trial by 12.5 s. Stimulus intensities were presented in four alternating descending and ascending series (step size, 5 μA), with five trials presented at each current intensity step. The current threshold for each series was defined as the midpoint between two consecutive intensity steps that yielded three or more positive responses and two consecutive intensity steps that yielded three or more negative responses. The overall ICSS threshold for the session was defined as the mean of the current thresholds from the four alternating series. To assess performance effects (e.g., motor disruption), response latencies (time between onset of the non-contingent stimulus and a positive response) were averaged across all trials in which a positive response was made.

Somatic withdrawal signs. Rats were placed in a clear plastic circular chamber and recorded with a digital camera for 10 min. Recordings were later scored for somatic signs by a blinded trained observer using a validated checklist (Gellert & Holtzman, 1978; Schulteis et al., 1994). Individual categories of withdrawal signs included eye blinks (5-9: 1 pt; 10+: 2 pts), wet dog shake (1-2: 2 pts; 3+: 4 pts), escape jumps (1-4: 1 pt; 5-9: 2 pts; 10+: 3 pts) , abdominal constrictions (2 pts/each), swallowing movement (2 pts), facial fasciculations (2 pts), abnormal posture (3 pts), ptosis (2 pts), penile grooming (3 pts), chromodacryorrhea (5 pts), salivation (7 pts) and diarrhea (2 pts). The somatic sign score was determined by adding all points across all categories of withdrawal signs.

Supplementary Results

Attrition

Several animals (see Results for group size of the MOR + NX group during each phase of the MSA protocol) were lost to attrition during the course of the protocol due to loss of ICSS headcap, loss of stability of ICSS thresholds, failure to acquire MSA, loss of catheter patency, health issues, or other problem. Data for these animals are analyzed for those phases they completed.

Baseline ICSS measures during acute dependence

Baseline ICSS thresholds and response latencies did not differ between groups during either precipitated or spontaneous withdrawal testing during the acute dependence phase (Table S1).

Locomotor activity

One-way ANOVA on total distance travelled during the locomotor activity session in the MOR + NX and control groups (MOR + SAL, SAL + NX, SAL + SAL) indicated no effect of group (Fig S1A). An additional one-way ANOVA of within-session activity in the MOR + NX group indicated a main effect of time ($F(3.417, 95.66) = 64.07$, $p < 0.0001$). Activity was highest during the first 30 minutes of the 2-hour session (Fig S1B), consistent with previous findings from our lab and others (e.g., Study 1; Piazza et al., 1989). Distance travelled did not correlate with any subsequent MSA measure (all $p \geq 0.11$).

Comparison of MSA in the MOR + NX and control groups

Rats in the MOR + NX group and control groups did not differ on most measures of MSA. Two-way repeated measures ANOVA on infusion rates between the MOR + NX and the control groups during the first 10 days of acquisition indicated a significant overall

effect of session ($F (9, 468) = 2.20$, $p = 0.02$) but no effect of group or interaction between group and session (Figure S2A). Two-way ANOVA on group and FR during demand testing showed a significant main effect of FR ($F (6, 246) = 152$, $p < 0.001$) and interaction between group and FR ($F (18, 246) = 2.144$, $p = 0.005$). but no main effect of group (Figure S2B). The MOR + NX had significantly lower infusions at FR 2 and FR 3 compared to the SAL + SAL group (all $p < 0.01$). However, a one-way ANOVA comparing elasticity of demand (α) (i.e., the primary outcome) indicated no significant difference between groups (Figure S2C). The MOR + NX and control groups also did not significantly differ in three secondary behavioral economic measures: intensity of demand (Q_0), maximal response output (O_{max}) and the unit price at which maximal response output occurred (P_{max}) (Table S2). Additionally, the groups did not differ in infusions during extinction, as a two-way repeated measures ANOVA revealed a significant overall effect of session ($F (10, 410) = 79.53$, $p < 0.0001$) (Figure S2D) but no effect of group or group x session interaction. Finally, two-way ANOVAs showed a main effect of reinstatement condition on reinstatement scores during morphine- and cue-induced reinstatement ($F (1.291, 51.65) = 28.95$, $P < 0.0001$) and yohimbine- and cue-induced reinstatement ($F (1.747, 65.79) = 22.68$, $P < 0.0001$), with responses significantly elevated during all drug (morphine or yohimbine) and/or cue conditions compared to the VEH + CUE control condition (Figure S2E-F). However, there was no significant main effect of group or interaction during either morphine- and cue-induced reinstatement testing or yohimbine- and cue-reinstatement testing.

Additional behavioral economics measures

Table S2 shows additional parameters from the exponential demand curve, However, these additional measures (Q_0, O_{max} or P_{max}) from demand testing did not significantly correlate with either ICSS thresholds or somatic withdrawal signs during naloxone-precipitated or spontaneous withdrawal in the Mor + NX group during the acute dependence phase.

Late-stage dependence

Precipitated withdrawal: ICSS. Baseline ICSS thresholds and response latencies for late-stage precipitated withdrawal did not differ between groups (Table S3). Two-way ANOVA on ICSS thresholds during precipitated withdrawal revealed a significant main effect of group ($F(3, 22) = 6.2$, $p = 0.003$), but no significant effect of session or interaction. Holm-Sidak's multiple comparison showed significantly higher ICSS thresholds in the MOR + NX group compared to the SAL + SAL control group during all sessions (all $t(22) \geq 3.71$, all $p < 0.05$) (Figure S3A). There was no significant effect of group, session, or group x session interaction on ICSS latencies during precipitated withdrawal (data not shown).

Spontaneous withdrawal: ICSS. Baseline ICSS thresholds and response latencies did not differ between groups (Table S3). ICSS thresholds during hours 2 (agonist effect) and hours 6 – 98 (withdrawal period) of spontaneous withdrawal did not differ between the two groups receiving MOR (MOR + NX versus MOR + SAL groups) or SAL (SAL + SAL versus SAL + NX groups). Data from these groups were therefore combined into a single MOR ($n = 15$) and SAL ($n = 5$) group. Welch's corrected t test showed no significant difference in ICSS thresholds between MOR ($118.4 \pm 14.03\%$) and SAL ($102.6 \pm 2.58\%$)

groups during the 2-hour session, indicating an absence of effects of MOR itself on ICSS. Two-way ANOVA on ICSS thresholds during spontaneous withdrawal 6-98 hours after morphine injection revealed no significant main effect of time, group (morphine vs. saline) or interaction (Figure S3B). However, comparison of peak ICSS threshold values between 6 hours and 98 hours (regardless of the time point at which they occurred) differed significantly between the morphine (121.7 ± 4.78%) and saline (106.8 ± 2.04%) groups (Welch-corrected $t(17.54) = 2.85$, $p = 0.01$). No significant difference in ICSS response latencies was observed between groups 2 hours after injection (agonist effect) or 6-98 hours after injection (withdrawal effect) (data not shown).

Precipitated withdrawal: Somatic signs. One-way ANOVA revealed a significant overall difference in somatic signs scores between groups during the 5th session of precipitated withdrawal during late stage dependence ($F(3, 23) = 4.57$, $p = 0.01$). However, Dunnett's multiple comparison indicated that none of the groups differed significantly from the SAL + SAL group (Figure S3C).

Spontaneous withdrawal: Somatic signs. Somatic signs did not differ between the two groups receiving MOR or between the two groups receiving SAL. Data from these groups were therefore combined into single MOR (n = 15) and SAL (n = 5) conditions, respectively. Total somatic signs in the MOR condition were significantly higher than in the SAL condition (Welch-corrected $t(21.44) = 2.19$, $p = 0.04$) (Figure S3D).

Correlations in the MOR + NX group during late-stage dependence

ICSS thresholds or somatic signs scores during late-stage dependence during precipitated or spontaneous withdrawal did not correlate with any MSA measure, except

for a significant correlation between higher composite z-score for ICSS thresholds during precipitated withdrawal and higher infusions during extinction (i.e., *greater* resistance to extinction; $r = 0.61$, $p = 0.04$).

Comparison of withdrawal severity during acute dependence and late-stage dependence in rats that completed both phases suggested that these measures were largely independent. Thus, ICSS thresholds during precipitated or spontaneous withdrawal during late-stage dependence did not correlate with these same ICSS measures during acute dependence (all r: $-0.16 \leq r \leq -0.04$, all $p \geq 0.57$). Somatic signs during spontaneous withdrawal correlated significantly between late-stage dependence and acute dependence ($r = 0.73$, $p = 0.007$), but this correlation was not observed during precipitated withdrawal ($r = 0.13$, $p = 0.45$).

Table S1

Mean (±SEM) ICSS thresholds (in μA) and response latencies (in sec) in experimental groups during baseline sessions during acute dependence testing.

	Naloxone-precipitated Withdrawal		Spontaneous Withdrawal	
	Threshold (μA)	Latency (sec)	Threshold (μA)	Latency (sec)
MOR+NX	131.5 ± 13.37	2.62 ± 0.10	132.5 ± 13.99	2.62 ± 0.09
MOR+SAL	123.3 ± 12.45	2.69 ± 0.20	127.0 ± 12.80	2.59 ± 0.13
SAL+NX	128.8 ± 15.50	2.50 ± 0.17	133.3 ± 17.90	2.60 ± 0.20
SAL+SAL	142.4 ± 25.14	2.65 ± 0.11	141.1 ± 25.31	2.71 ± 0.14

Table S2

Exponential demand curve parameters for individual subjects. Note: the parameter k (range of consumption) is set to 1.8 log units.

Subject	α	Q_0	P_{max}	O_{max}	R^2
MOR + NX					
1	0.0084	4.1	9.4	12.2	0.66
2	0.0047	11	6.2	21.9	0.81
3	0.0087	3.6	10.3	11.8	0.64
4	0.0039	19	4.4	26.4	0.85
5	0.0034	4.8	19.8	30.3	0.65
6	0.0011	3.7	79.3	93.5	0.7
7	0.0025	6.4	20.2	41.2	0.81
8	0.0052	10	6.2	19.8	0.65
9	0.0055	3.5	16.8	18.7	0.75
10	0.0025	4.2	30.7	41.2	0.72
11	0.0051	5.4	11.7	20.2	0.5
12	0.0021	7.6	20.2	49	0.89
13	0.0022	9	16.3	46.8	0.66
14	0.0053	3	20.3	19.4	0.68
15	0.00065	5.2	95.5	158.3	0.79
16	0.0017	10	19	60.5	0.79
17	0.0079	7.4	5.5	13	0.95
18	0.0023	7.4	19	44.7	0.79
19	0.0027	19	6.3	38.1	0.79
20	0.0012	5.1	52.7	85.7	0.9
21	0.0028	12	9.6	36.7	0.89
22	0.0037	8	10.9	27.8	0.84
23	0.0016	4.6	43.9	64.3	0.95
24	0.0016	3.6	56	64.3	0.93
25	0.00078	5.1	81.1	131.9	0.98
Mean	**0.003501**	**7.31**	**26.85**	**47.11**	**0.78**
SEM	**0.000465**	**0.87**	**5.22**	**7.37**	**0.02**
MOR + SAL					
1	0.0027	5	23.9	38.1	0.89
2	0.0012	6.3	42.7	85.7	0.89
3	0.0017	4.5	42.2	60.5	0.64
4	0.0038	4.7	18.1	27.1	0.68
5	0.00084	6.6	58.2	122.5	0.93

6	0.0016	8.9	22.7	64.3	0.99
7	0.0025	5.6	23.1	41.2	0.92
Mean	**0.002049**	**5.94**	**32.99**	**62.77**	**0.85**
SEM	**0.000384**	**0.58**	**5.61**	**12.39**	**0.05**
SAL + NX					
1	0.002	4.3	37.5	51.4	0.96
2	0.002	5.8	27.8	51.4	0.91
3	0.0039	13	6.4	26.4	0.96
4	0.0017	9.3	20.4	60.5	0.57
5	0.0053	6.7	9.1	19.4	0.9
6	0.0023	4.4	31.9	44.7	0.73
Mean	**0.002867**	**7.25**	**22.18**	**42.30**	**0.84**
SEM	**0.000582**	**1.37**	**5.11**	**6.53**	**0.06**
SAL + SAL					
1	0.0028	7.7	15	36.7	0.94
2	0.0016	13	15.5	64.3	0.85
3	0.0019	10	17	54.1	0.96
4	0.0046	7.1	9.9	22.4	0.58
5	0.0015	6.2	34.7	68.6	0.71
6	0.0018	13	13.8	57.2	0.86
7	0.0026	5	24.8	39.6	0.96
8	0.0026	6.8	18.3	39.6	0.94
Mean	**0.002425**	**8.60**	**18.63**	**47.81**	**0.85**
SEM	**0.000357**	**1.08**	**2.74**	**5.57**	**0.05**

Table S3

Mean (±SEM) ICSS thresholds (in μA) and response latencies (in sec) in experimental groups during baseline sessions during late-stage dependence testing.

	Naloxone-precipitated Withdrawal		Spontaneous Withdrawal	
	Threshold (μA)	Latency (sec)	Threshold (μA)	Latency (sec)
MOR+NX	134.3 ± 23.12	2.53 ± 0.11	120.6 ± 23.17	2.56 ± 0.09
MOR+SAL	125.1 ± 24.33	2.45 ± 0.12	122.6 ± 24.15	2.43 ± 0.08
SAL+NX	123.5 ± 17.16	2.52 ± 0.24	113.6 ± 30.19	2.29 ± 0.39
SAL+SAL	102.0 ± 20.27	2.22 ± 0.34	111.8 ± 27.11	2.27 ± 0.58

Table S4

Mean (±SEM) somatic sign scores in experimental groups during acute dependence naloxone-precipitated withdrawal testing. Each individual sign is shown in addition to the total. Data for escape jumps, salivation and diarrhea are not shown because these signs were not observed in any group.

	Eye Blinks	Wet Dog Shakes	Abdominal Constrictions	Swallowing Movements	Facial Fasciculations	Abnormal Posture	Ptosis	Penile Groom	Chromoda-cryorrhea	Total
MOR+NX	0.96±0.13	1.00±0.24	0.57±0.20	1.50±0.17	1.79±0.12	0.43±0.20	0.43±0.16	1.29±0.29	0.36±0.25	8.32±0.62
MOR+SAL	0.00±0.00	0.22±0.22	0.00±0.00	0.22±0.22	1.11±0.35	0.00±0.00	0.00±0.00	1.67±0.53	0.00±0.00	3.22±0.40
SAL+NX	1.00±0.15	0.00±0.00	0.20±0.20	0.80±0.33	0.80±0.33	0.60±0.40	0.20±0.20	0.30±0.30	0.00±0.00	3.90±0.81
SAL+SAL	0.89±0.26	0.89±0.35	0.22±0.22	1.33±0.33	0.89±0.35	0.00±0.00	0.00±0.00	1.33±0.53	0.00±0.00	5.56±0.71

Table S5

Mean (±SEM) somatic sign scores in experimental groups during acute dependence spontaneous withdrawal testing. Each individual sign is shown in addition to the total. Data for escape chromodacryorrhea, jumps, salivation and diarrhea are not shown because these signs were not observed in any group.

	Eye Blinks	Wet Dog Shakes	Abdominal Constrictions	Swallowing Movements	Facial Fasciculations	Abnormal Posture	Ptosis	Penile Groom	Total
MOR+NX	1.15±0.15	0.46±0.20	0.69±0.35	1.31±0.19	1.54±0.17	0.23±0.16	0.00±0.00	0.81±0.27	6.19±0.77
MOR+SAL	0.78±0.28	0.67±0.47	0.22±0.22	0.44±0.29	1.78±0.22	0.00±0.00	0.00±0.00	1.67±0.53	5.56±1.17
SAL+NX	0.67±0.17	0.44±0.29	0.00±0.00	0.22±0.22	0.89±0.35	0.00±0.00	0.22±0.22	1.67±0.53	4.11±0.92
SAL+SAL	0.90±0.23	0.20±0.20	0.40±0.27	0.80±0.33	0.80±0.33	0.30±0.30	0.20±0.20	0.60±0.40	4.20±0.61

Figure S1: (A) Mean (± SEM) total distance travelled (in cm) during the 2-hr locomotor test in each experimental group; (B) Mean (± SEM) distance traveled in 5-minute blocks during locomotor testing in the MOR + NX group.

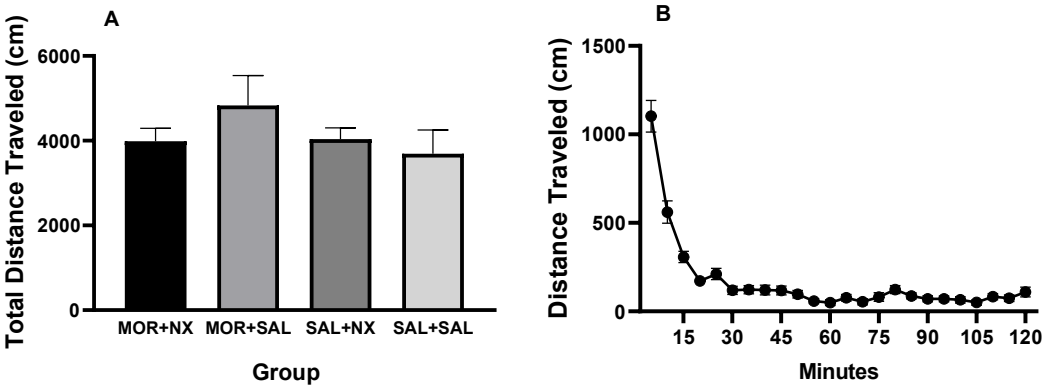


Figure S2: (A) Mean (± SEM) infusions during MSA acquisition for the MOR + NX and control groups. (B) Mean (± SEM) infusions at each FR during demand testing. ** Different from SAL + SAL group at that FR, $p < 0.01$. (C) Exponential demand curve describing morphine consumption as a function of unit price for rats as a group for each of the 4 groups. (D) Mean (± SEM) number of infusions during baseline and MSA extinction over 10 sessions. (E and F) Mean (± SEM) reinstatement scores during morphine- and cue-induced reinstatement (E) and yohimbine- and cue-induced reinstatement (F).

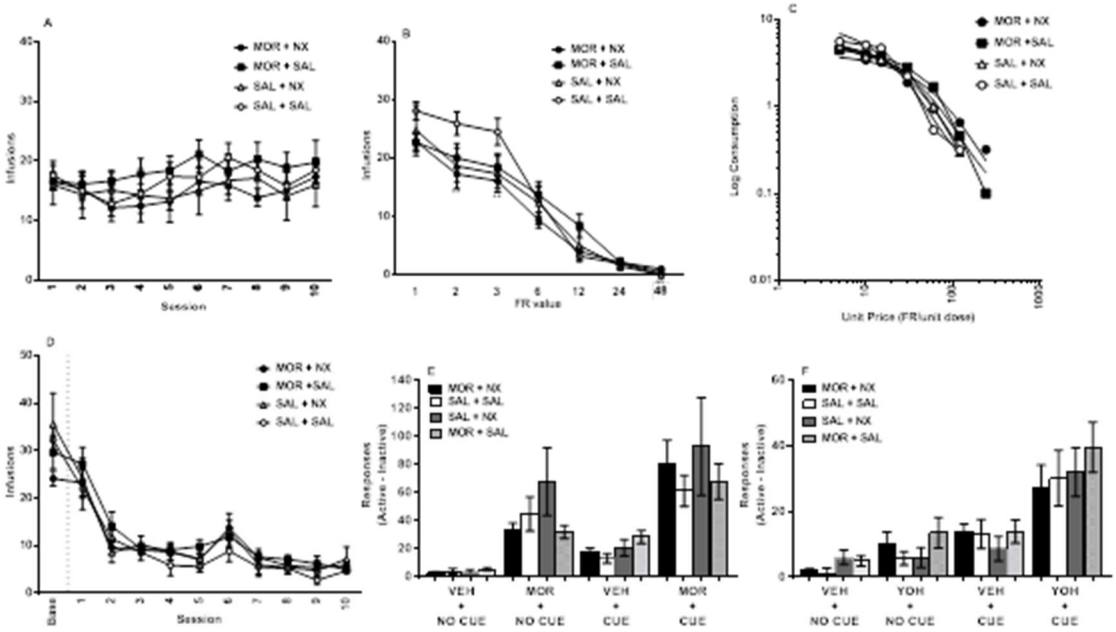


Figure S3: Mean (± SEM) ICSS thresholds (expressed as percent of baseline) during late-stage naloxone-precipitated withdrawal (A) and spontaneous withdrawal (B). * Different compared to SAL + SAL group at that session, $p < 0.05$. Mean (± SEM) somatic signs in groups on the 5th day of late-stage dependence precipitated withdrawal (C) and 26 hours after injection during late-stage dependence spontaneous withdrawal (D). *** Significant effect of group, $p < 0.001$. * Different from SAL condition, $p < 0.05$.